CELL-FREE TRANSMISSION IN ADULT SWISS MICE OF A DISEASE HAVING THE CHARACTER OF A LEUKEMIA*,‡

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PLATES 24 TO 27

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Since the classic works of Ellermann and Bang on the avian leukosis (1) and of Rous on the chicken sarcoma (2), the concept that viral agents may play a role in the induction of many neoplastic diseases has been seriously considered. Shope's discovery of the infectious papilloma agent of rabbits (3) and Bittner's of the carcinoma-inducing agent in the milk of certain strains of mice (4) have given further credence to this theory.

In recent years there has been much evidence pointing to the viral causation of leukemia in mice. Gross (5–7) has reported that many newborn mice of a low leukemic strain, when inoculated with cell-free material obtained from leukemic organs of either AK or C_{58} high leukemic strains of mice, develop leukemia, sarcomas, or parotid gland tumors upon reaching maturity. Stewart (8, 9) and Woolley (10) have carried out experiments confirming the presence of such oncogenic agents in AK leukemia extracts. Schwartz *et al.* (11) recently reported the accelerated development of leukemia in approximately 50 per cent of young adult AK mice 2 to 12 weeks after their inoculation with filtrates prepared from the brains of leukemic AK mice.

The present communication describes a filterable agent obtained from the spleen of a leukemic Swiss mouse and the malignant proliferative disease of the hematopoietic system which it consistently produces on serial transmission to adult mice.

Origin of the Agent

In the course of examination of the Ehrlich ascites tumor with the electron microscope it was noted that the cytoplasm of some of the tumor cells contained particles of constant diameter in close array (12). The similarity of

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these particles to those seen in virus-infected cells prompted an investigation of the possible viral etiology of the tumor. It was during this study that the agent now to be described was isolated.

The Ehrlich ascites carcinoma was originally obtained from Dr. K. Suguira of the Sloan-Kettering Institute. It is maintained by routine passage in Swiss mice by the intraperitoneal injection at 14 day intervals of diluted ascitic fluid containing approximately one million tumor cells. A cell-free extract of the tumor was prepared as follows.

Fourteen days after the injection of mice with the Ehrlich ascites tumor, the ascitic fluid from these animals was collected and centrifuged at low speed (500 to 1000 R.P.M.) for 10 minutes. The supernatant was removed and discarded. The sedimented cells were ground with sand in a mortar and suspended in buffered Locke-Ringer's solution.[1] With the aim of freeing the suspension of intact cells, the ground tumor suspension (11 ml.) was centrifuged in a Spinco model L at 40,000 R.P.M. for 2 hours and the upper third of the supernatant in the Spinco tubes was removed without disturbing the sedimented pellet. One tenth of 1 ml. of this supernatant fluid was injected subcutaneously into each of thirty Swiss mice less than 24 hours of age. After a 14 month period of observation, during which all the animals remained apparently healthy, these mice were sacrificed. Microscopically and in the gross there was no evidence of the Ehrlich tumor in any of the thirty animals autopsied. However, it was noted at autopsy that six of the thirty mice had enlarged spleens and livers. Each of the six spleens was removed aseptically, finely minced with scissors, and suspended individually in Locke-Ringer's solution. Each suspension was injected intraperitoneally in 0.2 ml. amounts into six groups of five adult Swiss albino mice each, since no suckling mice were available at the time. Four of the groups remained negative, but in two marked enlargement of the spleen and liver took place in three of the five mice injected in each group. Passages from one of these groups were discontinued after the third transfer generation. Passages from the other have furnished the subject of this report. It is now in its twenty-sixth serial transfer.

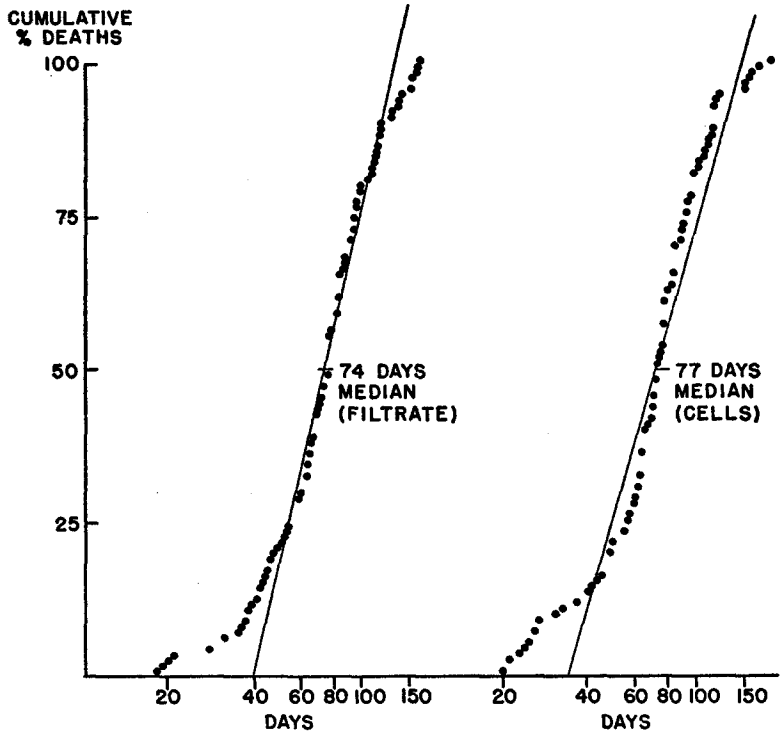
Characteristics of the Disease Studied

Two to 3 weeks after the intraperitoneal injection of a 10 per cent suspension of affected spleen, or of a filtrate of the suspension, made as described further on, mice develop spleens so enlarged that they can be detected by abdominal palpation. The animals show no other obvious signs for 2 to 3 months and appear healthy until a few days before death. At this time they become inactive and emaciated and the fur roughens. The proportion of deaths varies from experiment to experiment but is usually in excess of 85 per cent. The time at which death occurs after the injection of either a suspension of about ten million whole cells or a filtrate of the suspension is plotted in Text-fig. 1. There were 110 mice in each series plotted.

As can be seen, the range in the time of death is from 30 to 100 days, with an occasional animal dying earlier or surviving longer. The median day of death, nearly the same in both groups, is 77 days for suspension-injected animals and 74 days for those which received filtrates.

[1] Locke-Ringer's solution contains 6 mg. of glucose per ml., 50 μ. of penicillin per ml., and 2 mg. of streptomycin per ml.

The disease produced is identical as concerns incubation period and hematological changes, whether intact cells or filtrates are injected. The white blood count—which has been taken at weekly intervals—generally remains under 50,000 cells per mm.^{3} in the first 4 weeks following inoculation. A sharp rise occurs between the 5th and 7th week and may reach over 300,000 cells per mm.^{3} terminally. At this time there is also a marked decrease in the red blood cell count. Representative red and white cell counts are listed in Table I. The peripheral blood contains large mononuclear cells with a rounded, lobed, or horseshoe shaped nucleus. (Figs. 1



TEXT FIG. 1. Survival time of 110 mice after inoculation with filtrate or cells of infected spleen. Each black dot stands for a mouse. Cumulative percentage of deaths is plotted against post inoculation days.

and 2). These cells are extremely fragile and often appear smudged in smears. Their origin is still undetermined. Hematologists who have examined them differ as to whether they are of the granulocytic or monocytic series. Nucleated red blood cells, generally from 1 to 10 per 100 white blood cells, also appear in the peripheral blood in the terminal stages of the disease.

Over 400 mice have been examined to date. At autopsy of mice dead of the disease the liver and spleen are greatly enlarged. The spleen, for example, may weigh from 2 to 8 gm., in comparison with a normal spleen weight of 0.2 gm. or less. Massive peritoneal hemorrhage is found in approximately one-third of the animals, and in about one-half of the mice in which hemorrhage is found the spleen has ruptured. The lymph nodes are increased to two to three times normal size and are firm. Other organs appear normal in the gross. Fig. 3 illustrates the

characteristic gross findings in a mouse which died of the disease. A normal mouse is shown for comparison.

Microscopic examination of the organs of the diseased mice revealed that the principal changes were in the bone marrow, spleen, liver, and the peripheral blood. These organs were markedly infiltrated with abnormal cells. Prominent in the infiltrations was a large mononucleated cell generally resembling that in the blood, its major portion consisting of a more or less rounded pale nucleus having a fine chromatin pattern. Round the periphery was a thin rim of dark blue cytoplasm. In the early stages of the process, about 1 to 2 months after injection of a suspension or filtrate of spleen, decalcified sections of the sternal marrow showed these cells to be present in focal collections surrounded by normal marrow cells. Terminally, they were found diffusely replacing a major portion of the marrow elements (Figs. 4 to 6). In the spleens these cells were present in both follicles and red pulp. In nearly all instances of

TABLE I

Representative White and Red Blood Counts of Infected Mice

Cell suspension*				Cell-free filtrate‡			
Mouse No.	Post Inoculation	WBC (10^3)	RBC (10^6)	Mouse No.	Post Inoculation	WBC (10^3)	RBC (10^6)
	days				*days*		
27-A-4	27	36.7	9.2	I-15-4	43	43.4	13.4
8-A-2	53	79.4	9.3	I-5-2	45	98.8	10.4
12-A-5	59	65.2	10.2	XV-5-1	50	80.6	14.3
12-A-2	62	200.4	5.1	XVI-1-2	63	136.8	8.7
5-A-3	66	158.6	6.8	XV-1-5	67	337.4	5.5
21-Al-6	88	184.3	8.6	IX-1-2	81	230.0	4.7
Controls (8)		16.1	11.2				

* 0.2 ml. supernatant of 10 per cent suspension, i.p.

‡ 0.2 ml. of 10 per cent filtrate, i.p.

marked involvement the demarcation of the follicles and pulp was lost and almost the entire spleen was replaced by these abnormal cells (Figs. 7 and 8). The liver was involved in a similar manner (Figs. 9 to 12). The sinusoids were widely distended by immense numbers of the large mononuclear cells and there was marked atrophy of the hepatic cords. In some areas the organ could barely be recognized as such because of the extensive infiltration. In a few instances focal collections of cells of the same sort were found in the kidney (Fig. 13). In the lungs (Fig. 14) there was a widespread infiltration of the alveolar walls with the characteristic mononuclear cells. The alveolar spaces were diminished in size and occasional tumor cells could be seen lying free in the blood vessels. The lymph nodes showed only moderate involvement by these cells, and massive enlargement of them did not occur. In all the involved organs, as well as the peripheral blood, varying numbers of normoblasts were seen. No significant changes have been noted in other organs.

An interesting manifestation of the disease was the occasional appearance of ascites in addition to the other pathological changes. An attempt was made to start up an ascites line of the disease.

Five ml. of slightly turbid fluid were withdrawn which on examination contained the same type of large mononuclear cell observed in the peripheral blood of the mice terminally. These cells were morphologically distinct from the cells of the Ehrlich tumor. The ascites has appeared as early as 1 month after injection of these cells, and the disease was transferred by means of it for five serial passages. At autopsy of mice with ascites the peritoneal linings did not appear abnormal nor were there any patches of solid tumor. After the fifth passage, formation of ascites failed to occur, yet the disease had been transmitted as evidenced by the appearance of enlarged livers and spleens at death.

Transmission of the Disease

Two serial passages of the disease are being maintained concurrently in adult Swiss mice. One passage is made by transferring intact cells and the other by transferring cell-free filtrates of infected spleens.

Cell Transfers.—For the first transfers the mice were from 2 to 7 months of age at the time of injection. Later mice approximately 2 months of age and with a uniform weight of 16 to 20 gm. were used, and they yielded a higher percentage of positives.

Routine cell passages are carried out by preparing a 10 per cent suspension of finely minced infected spleen in Locke-Ringer's solution. After low speed centrifugation to remove coarse particles, 0.2 ml. of the supernatant fluid is injected intraperitoneally into mice. The number of cells in the inoculum is generally about 10 million. It is of interest to note that inoculation of only 100 or 1,000 cells has failed to produce the disease during a 10 month observation period.

Cell-Free Transfer.—The first passage presumably free from cells was carried out by means of an extract of spleens of the third transfer generation. Since then serial transfers have been made with filtrates.

For these passages the spleens are ground in a mortar and a 10 per cent suspension in Locke-Ringer's solution is prepared. After low speed centrifugation the supernatant is removed. For each 10 ml. of supernatant fluid 0.2 ml. of a 24 hour nutrient broth culture of *Escherichia coli* is added. The supernatant *E. coli* mixture is then filtered through Selas 03 or Berkefeld N filter candles. The agent has also been found to pass through gradocol membranes[2] with a pore size of 220 mμ. Samples of the material before and after filtration are inoculated into tubes of nutrient broth to provide a check on the retention of bacterial cells by each filter. The filtered fluid—free from *E. coli*—readily transmits the disease. Serial cell-free passages are kept up by intraperitoneal injection of 0.2 ml. of newly prepared spleen filtrate.

The results of serial transmissions through twenty-six passages by means of filtered fluid and cells respectively are summarized in Table II. As with the median day of death (Text-fig. 1), there is a marked similarity in the number of positive animals, whether inoculated with intact cells or with cell-free filtrate. The increasing percentage of positive takes in the later passages, as compared with the earlier ones might be considered a manifestation of an increasing "adaptation" of the filterable agent to the host, as frequently occurs

[2] Gradocol membranes were obtained from Dr. Fred Himmelweit, London.

with newly isolated viruses. However, the results may merely have been due to more uniformly susceptible groups of animals.

Another point of comparison was available in the titration of the infective agent present in the superantant fluid from a suspension of minced cells and

TABLE II

Results of Passages

Serial transmission of the disease by means of spleen cell suspensions or cell-free filtrates.

Material from:	Cell suspension			Cell-free filtrate		
	No. mice inoculated	No. positive	Positive	No. mice inoculated	No. positive	Positive
			per cent			*per cent*
Passages 1–10.....	129	72	55.8	94	63	67.0
Passages 11–20.....	119	99	83.1	125	106	84.8
Passages 21–26.....	70	63	90.0	59	53	89.8

TABLE III

*Titer of the Agent in Infected Spleen**

Dilution‡	Supernatant of cell homogenate		Filtrate of supernatant	
	No. mice inoculated	No. mice positive	No. mice inoculated	No. mice positive
10^{-1}	5	4	5	5
10^{-2}	5	3	5	5
10^{-3}	5	4	5	3
10^{-4}	5	2	5	0
10^{-5}	5	2	5	0
LD_{50}	$10^{-3.6}$		$10^{-3.2}$	

* Spleen from mouse sacrificed 21 days after inoculation.

‡ 0.2 ml. of each dilution, i.p.

a filtrate from the same material. Results of such a comparative titration are given in Table III. It will be seen that the LD_{50} with the minced cells was $10^{-3.6}$ and, with the filtrate, $10^{-3.2}$.

Routes of Inoculation.—The mice can be infected by the intraperitoneal, subcutaneous, intracerebral, and intramuscular routes. In mice inoculated by the three latter routes the onset of the disease is delayed several days, as com-

pared with those inoculated intraperitoneally, but the course remains essentially the same. No tumor forms at the site of inoculation, yet the generalized leukemia-like disease develops. This is not unique, since it is known that local growths in some mouse leukemias may be small or absent (13). Transmission by direct contact does not appear to take place; control mice in the same cage as those inoculated have remained in good health throughout the course of the disease. The disease can be transmitted to infant mice, using

TABLE IV

*The Transmission of the Disease by Inoculation of Homogenates of Various Organs Obtained from Infected Mice**

Organ‡	No. inoculated	No. positive	Positive
			per cent
Spleen	10	10	100
Whole blood	8	8	100
Kidney	10	10	100
Lymph nodes	10	8	80
Lung	10	8	80
Liver	10	7	70
Heart	4	2	50
Brain	10	4	40
Skin	5	2	40

* Sacrificed 16 days after inoculation.

‡ 0.2 ml. i.p. of supernatant of 10 per cent homogenate. Blood undiluted.

any of the methods described for adult mice, and death occurs within 2 to 3 months following inoculation as in adult animals. There is no marked dependence of transmissibility on age or sex of the mice.

Transmission with Tissues Other Than Spleen.—In two separate experiments the fact has been demonstrated that the disease can be transmitted with the cells of other organs besides the spleens from infected mice. The results of these experiments are presented in Table IV.

Sixteen days after inoculation three mice were bled from the heart to exsanguinate them in so far as feasible and then killed by dislocating the neck. The organs listed in the table were removed aseptically from each animal and those of the same sort were pooled for test. Each suspension was injected intraperitoneally in 0.2 ml. amounts. Preparations from kidney and

whole blood, as well as spleen, transmitted the disease to all of the inoculated mice. The suspensions from liver, lung, and node were 70 to 80 per cent positive, whereas those from brain, heart, and skin ranged between 40 to 50 per cent.

The experiments show only the distribution of infective material in the different organs, since the supernatants from suspensions were tested instead of filtrates.

Susceptibility of Other Strains of Mice besides the Swiss.—Seven strains of adult mice have been tested, and thus far only the Swiss and the DBA/2 have developed the disease. A line is being maintained in the latter strain as well

TABLE V

Stability of the Agent

Treatment	Material	No. inoculated	No. positive	Positive
				per cent
X-ray (50,000 r)	Whole spleen	20	17	85
Lyophylized (3 mos.)	Filtrate	10	10	100
4°C. (11 days)	"	10	7	70
−70°C. (6 mos.)	"	10	6	60
56°C. (30 min.)	"	10	0	0
Ether (overnight, 4°C.)	"	15	0	0
Formalin (1:200)	"	20	0	0

as in the Swiss. Adult animals of the PRI, $C_{57}B1/6$, A, C_3H, and $F_1(C_{58} \times$ BALB) strains proved resistant.

Stability of the Agent

A small series of experiments have been carried out to learn the stability of the agent to certain physical and chemical treatment. Of particular interest is the marked resistance of the agent to massive doses of irradiation lethal to tumor cells (14, 15).

Two equal portions of each of several infected spleens were pooled, weighed, cut into millimeter cubes, and placed in two Petri dishes containing moistened filter paper. One dish was set aside as a control, and the other irradiated. Two 180 kv. tubes were used, one above and one below the dish. After exposure to 50,000 r the irradiated and non-irradiated tissues were each ground and enough Locke-Ringer solution added to make a 10 per cent concentration, based on the original net weight. Mice were inoculated intraperitoneally with 0.2 ml. amounts of one suspension or the other. Both caused the usual disease and no difference what-

ever could be seen in the infectivity of the irradiated material as compared with the control. Pooled results of two experiments carried out in the same way are shown in Table V. Further experiments to find out the limits of resistance to radiation are in progress, and they indicate that splenic fragments exposed to 100,000 or 150,000 r retain infectivity. In these later studies spleens from mice with transplantable leukemia 82B, a stem cell leukemia which yields no virus, have served as an additional control. The viability of the 82B leukemia cells was destroyed by the doses of irradiation.

TABLE VI

Lack of Immunological Relationship between the Agent and LCM

A

*Neutralization Test**

	Normal serum		Anti-LCM serum	
	No. deaths/No. inoculated	Positive	No. deaths/No. inoculated	Positive
		per cent		*per cent*
Agent	20/20	100	18/19	94.7
LCM	20/20	100	0/20	0

* 0.03 ml. i.c. of serum-virus mixture after incubation at 37°C. for 1½ hours.

B

Protection Test

Inoculum	Control mice		LCM-immunized mice	
	No. deaths/No. inoculated	Positive	No. deaths/No. inoculated	Positive
		per cent		*per cent*
Agent*	14/15	93.3	17/19	89.6
LCM‡	20/20	100	0/19	0

* 0.03 ml. i.c. of 10^{-1} dilution of spleen filtrate.
‡ 0.03 ml. i.c. of 10^{-3} dilution of LCM-infected brain.

Other data on the stability of the agent are included in Table V. Filtrates of infected spleens were still active after storage at −70°C. for 6 months and for at least 11 days at 4°C. Filtrates of 10 per cent suspensions of infected spleen retain their infectivity when lyophilized and stored for 3 months, as shown when they are reconstituted with Locke-Ringer's solution and inoculated into mice.

The infectivity of the agent is destroyed by heating at 56°C. for 30 minutes, by overnight exposure to ether at 4°C., and by treatment with a 1:200 dilution of formalin.

In view of the experiences of Stewart and Haas (16) and of many virologists who have encountered lymphocytic choriomeningitis (LCM) as a latent virus in their experimental animals, it seemed desirable to find out whether there was any immunological relationship between LCM and the agent under discussion. This was done despite the fact that LCM gives rise to a disease wholly different from that dealt with here (16).

Tests of two types were carried out. Forty mice were given an intraperitoneal inoculation of a 10 per cent brain suspension of the Armstrong[3] strain of LCM containing approximately three million intracerebral LD_{50} doses. Twenty other animals were set aside for normal controls. After 3 weeks the immunized animals were divided into four equal groups. Those of one group were bled to obtain immune serum for *in vitro* neutralization tests. Two groups were used for protection tests and the fourth group was held to observe whether any LCM-injected animals would show characteristics of the disease now under consideration.

For the *in vitro* neutralization test a filtrate of a 20 per cent brain suspension of LCM or a filtrate of a 20 per cent suspension of infected spleen was each mixed with an equal quantity of undiluted normal mouse serum or LCM immune serum. The mixtures were held for one and a half hours at 37°C., after which each was inoculated intracerebrally in 0.03 ml. amounts into groups of ten mice each. Deaths occurring within the first 24 hours were discarded from the results. The pooled results of two experiments are shown in Table VI (A). LCM antiserum failed to neutralize the splenic agent, while it completely neutralized LCM virus.

For the *in vivo* protection test a 10^{-3} dilution of LCM-infected brain (approcimately 30,000 ic. LD_{50}'s) was inoculated intracerebrally into LCM immune mice and ten normal mice of the same age. Similarly a 10 per cent dilution of splenic filtrate was inoculated intracerebrally into LCM-immune and normal mice. The pooled results of two experiments, presented in Table VI (B), confirm the neutralization test findings. The LCM-immune mice were fully susceptible to the splenic agent but resisted LCM infection. All surviving animals from both series of experiments are still being observed. Five months have passed with one series and three months with the other, and thus far the LCM-immunized mice, followed since the beginning of the experiments, have shown no evidence of leukemia or LCM.

DISCUSSION

A first question to be asked concerns the nature of the disease described: Is it a true leukemia? The pathological findings resemble those associated with leukemia instead of ordinary extramedullary myelopoiesis (17). The disease is characterized by marked proliferation of immature mononuclear cells which invade the spleen, liver, bone marrow, kidney, and lung, and appear in the peripheral blood. These large cells can be seen in various stages of mitosis in the organs they infiltrate. Terminally, the mice have greatly elevated white blood counts, are anemic, and have tremendously enlarged spleens and livers.

The second question concerns the agent producing the disease: What is its nature? Its viral character is indicated by the following evidence: (*a*) The disease can be transmitted serially by means of extracts passed through filters

[3] The Armstrong strain of LCM was obtained from the American Type Culture Association.

which hold back *E. coli*. (*b*) The agent causing it is resistant to an exposure to x-ray many times that needed to destroy normal cells of the same kind. (*c*) When dried, the agent retains its activity for long periods.

Graffi, Bielka, and Fey (18), and Schmidt (19), state that they had prepared cell-free filtrates from their strains of the Ehrlich ascites tumor which will induce leukemia if inoculated into newborn Agnes Bluhm mice. However, their findings differ from the ones described in this report in at least three respects: (*a*) The agent of the disease now under consideration has a much shorter period of latency. (*b*) It produces the disease in adult animals. (*c*) It can be passed serially in such animals.

Though the present agent was primarily recovered from a diseased Swiss mouse inoculated in infancy with material from the Ehrlich ascites tumor, this tumor itself has not appeared in over 400 mice examined after injection with the agent. Whether this was latent in the Ehrlich tumor cells or its presence together with the tumor was accidental is still not clear and must await further study. The possibility of the agent being the cause of a spontaneous disease which developed sometime during the 14 month observation of the original mouse should be considered.

SUMMARY

A disease with the characteristics of a leukemia has been found to be serially transmissible in adult Swiss mice by means of cell-free filtrates. Thus far, the disease has been transmitted through twenty-six serial passages with filtrates as well as cell suspensions. The agent readily passes through Selas 03, Berkefeld N, and gradocol membrane filters—these last having an average pore size of 220 mμ. Filtrates remain stable when stored for long periods at −70°C. or when lyophilized. Splenic tissue containing the agent, which was subjected to massive doses of x-ray (50,000 r),—far more than sufficient to kill the cells,—show undiminished infectivity. The agent is inactivated by heating to 56°C. for 30 minutes and by exposure to ether or formalin.

The disease can be transmitted to adult Swiss mice or DBA/2 mice, but not to adult PRI, C_3H, A, $C_{57}B1/6$, or $F_1(C_{58} \times BALB)$ mice. Intraperitoneal, subcutaneous, intracerebral, and intramuscular injections are all effective.

Grateful acknowledgment is made to Dr. Alice E. Moore and Dr. C. P. Rhoads for their encouragement and advice throughout this study, and to Dr. Stephen S. Sternberg for examining the histological sections from the infected mice.

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EXPLANATION OF PLATES

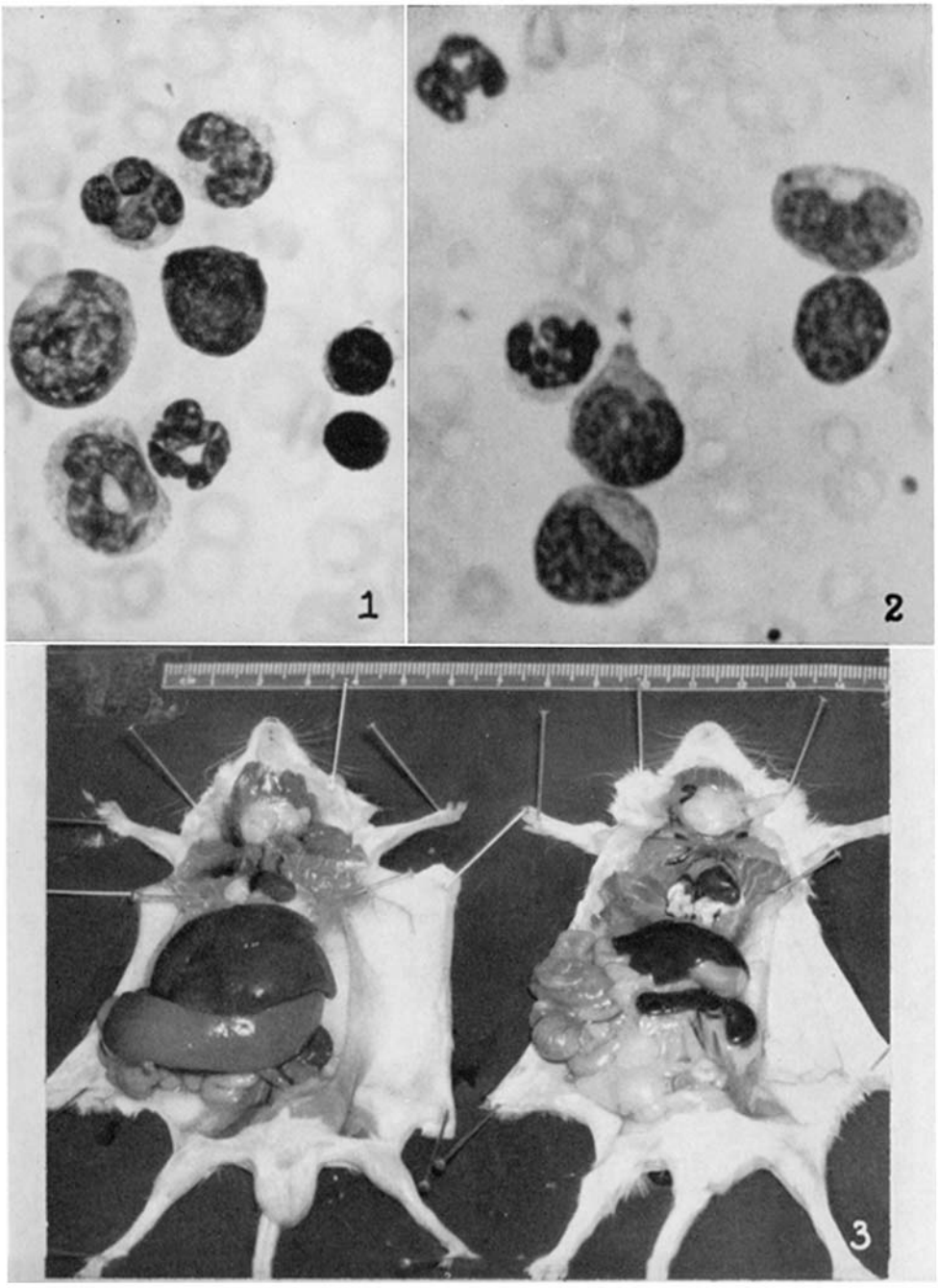
All sections were stained with hematoxylin and eosin, and the blood and impression smears with tetrachrome.

PLATE 24

FIG. 1. Peripheral blood of mouse bled on the 88th day after inoculation with the agent. The WBC was 184,350 cells/mm^3, and the RBC 8,860,000 cells/mm.^{3}. × 1350.

FIG. 2. Peripheral blood of mouse bled on the 81st day after inoculation. The WBC was 230,000 cells/mm.^{3}, and the RBC was 4,700,000 cells/mm.^{3}. Polymorphonuclear leukocytes, lymphocytes, and the abnormal mononuclear cells are present. × 820.

FIG. 3. The mouse on the left was found dead 57 days after inoculation. There is marked enlargement of liver and spleen. The mouse on the right is an uninjected control of the same age.



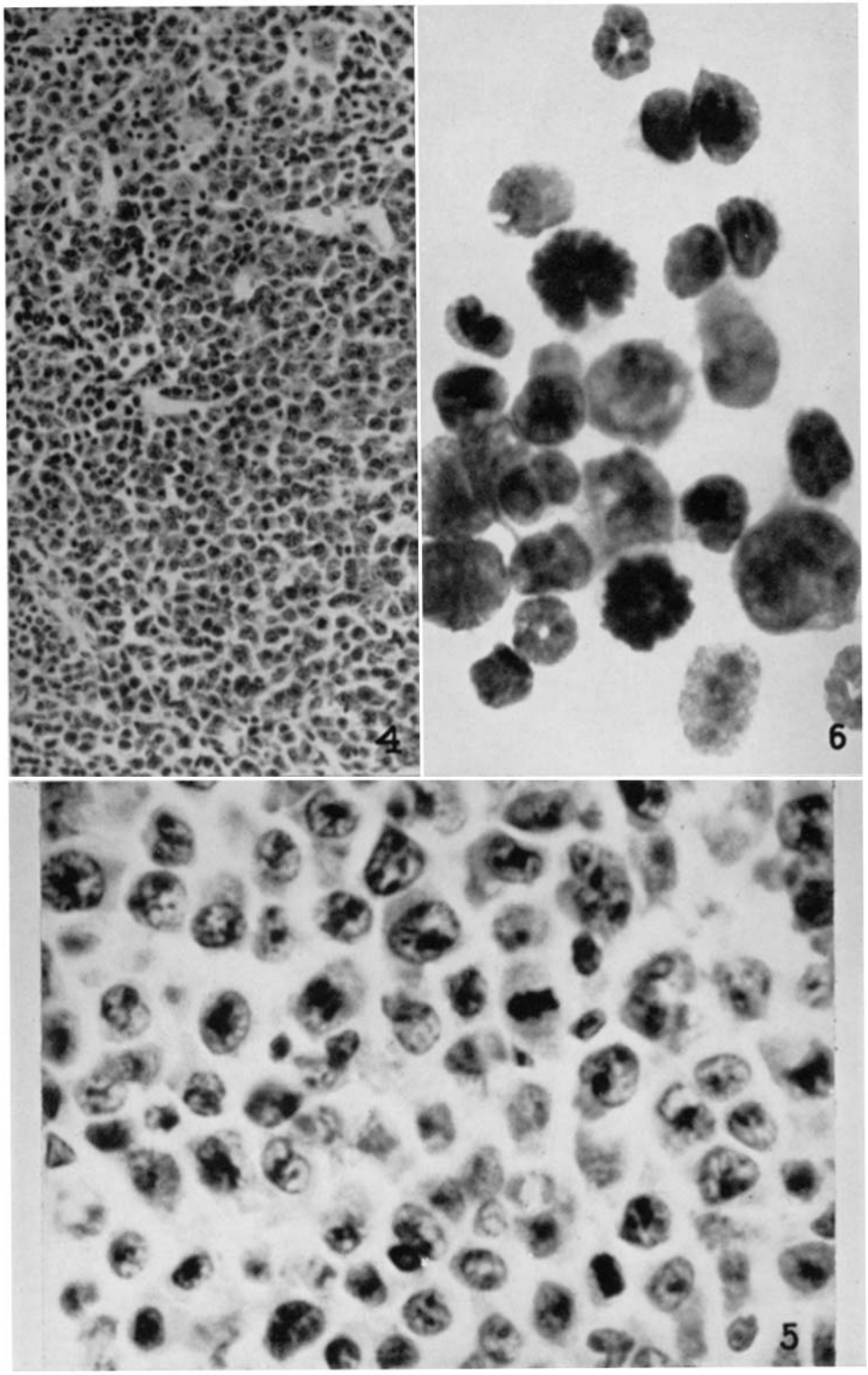
(Friend: Cell-free transmission of a mouse leukemia)

PLATE 25

FIG. 4. Sternal marrow of mouse whose blood picture is shown in Fig. 1. It is crowded with large mononuclear cells. × 200.

FIG. 5. Portion of the same at higher magnification, showing large mononuclear cells with vesicular nuclei. Mitotic figures are evident. × 920.

FIG. 6. Impression of femural marrow from the same mouse showing the mononuclear cells, some of which are in mitosis. × 1350.



(Friend: Cell-free transmission of a mouse leukemia)

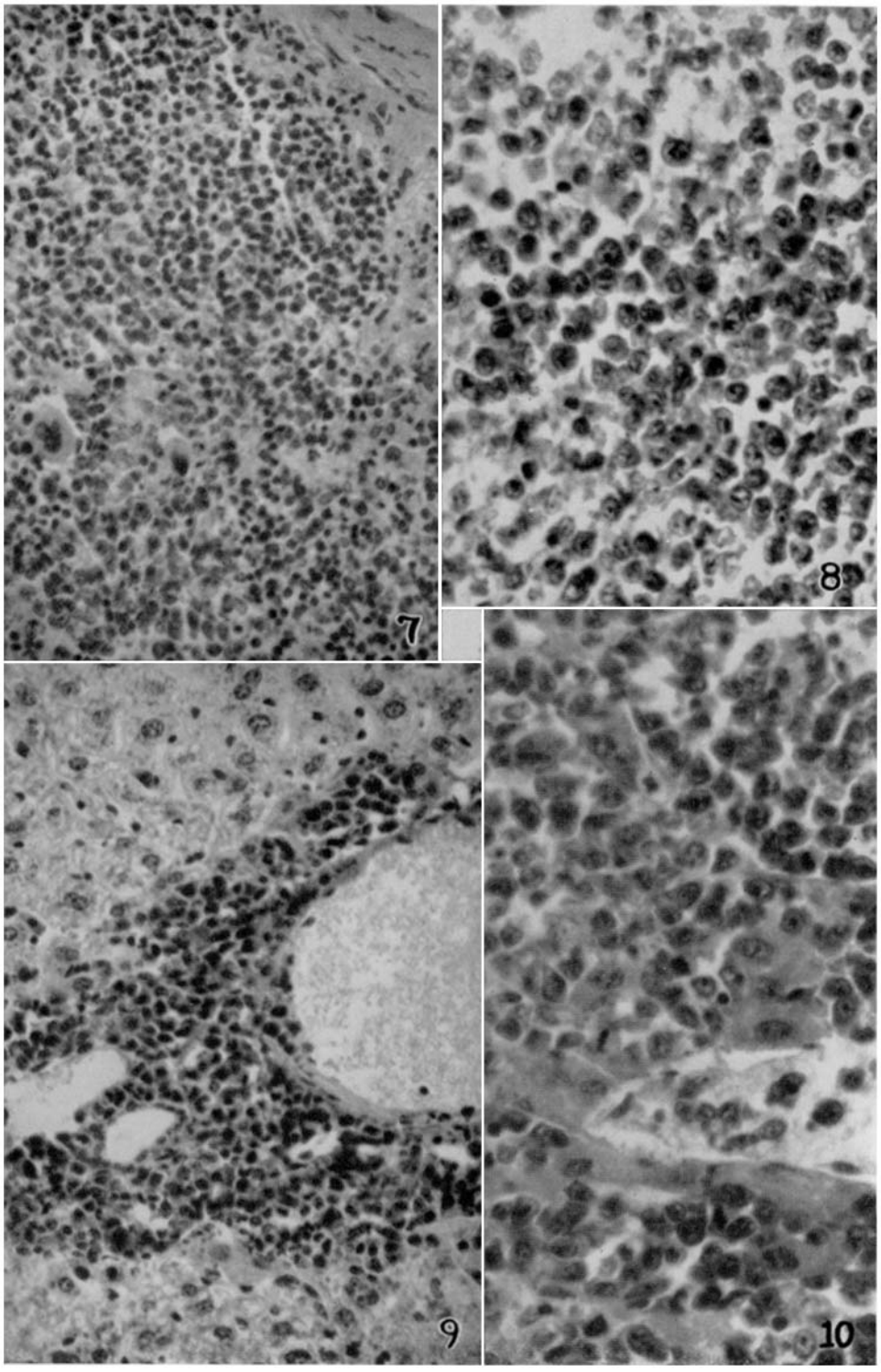
PLATE 26

FIG. 7. Spleen from the same mouse showing a loss of demarcation of the follicles and the red pulp. × 300.

FIG. 8. Part of the same at a higher power. × 400.

FIG. 9. Liver. Perivascular infiltration in the liver of a mouse sacrificed 60 days after inoculation. × 300.

FIG. 10. The same at a higher magnification. × 400.



(Friend: Cell-free transmission of a mouse leukemia)

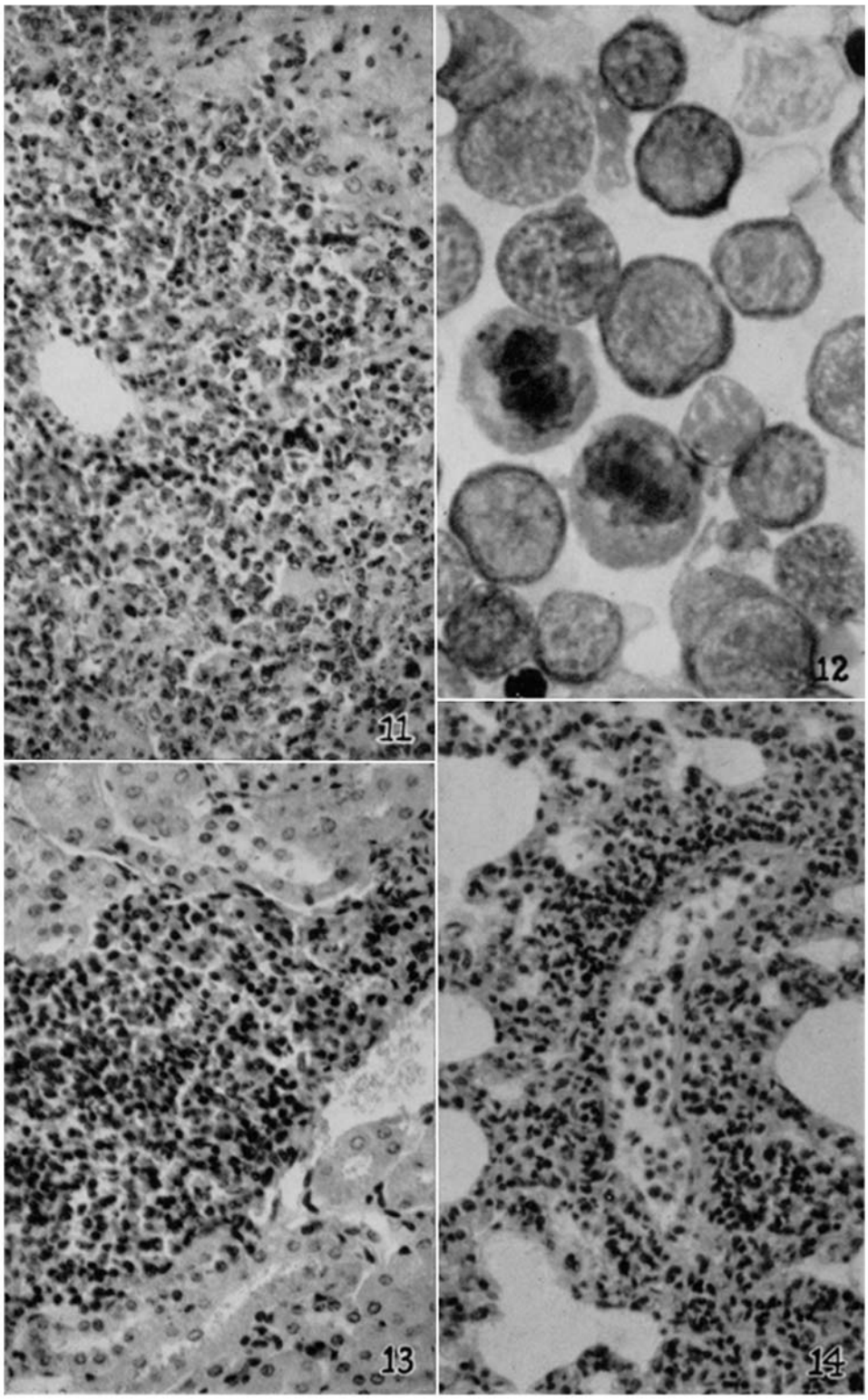
PLATE 27

FIG. 11. Liver. Greatly involved liver of mouse killed 88 days after inoculation whose spleen is shown in Fig. 7. There is massive infiltration and few of the hepatic cords remain. × 300.

FIG. 12. Impression of same liver showing numerous large mononuclear cells, two of them in mitosis, as well as part of two normoblasts. × 1350.

FIG. 13. Kidney: showing a focal collection of cells in the renal cortex. × 300.

FIG. 14. Lung: infiltration of the alveolar walls is to be seen and many free mononuclear cells lying in a blood vessel are seen. × 200.



(Friend: Cell-free transmission of a mouse leukemia)