High Susceptibility of 1 to 14 Days Old C3H Mice to "Passage A" Leukemic Filtrates.* (23722)

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In 1951 it was observed in our laboratory that mouse leukemia could be transmitted by cell-free filtrates to newborn mice of a susceptible strain(1,2). This observation was recently confirmed by Woolley(3), Furth(4), Dulaney(5), Hays(6), and their associates. Among the extracts prepared from Ak donors with spontaneous leukemia, some were highly active, others less potent, on inoculation tests(7,8.) Using one of the more active extracts, and passing it through several successive cell-free inoculations of newborn hosts, it was possible to obtain a highly potent agent designated "passage A"(8). Filtered extracts prepared from this leukemic passage strain, inoculated into less than 16 hours old C3H mice of the Bittner substrain(9), induced acute lymphatic leukemia in from 40 to 100% of the inoculated mice, and in some instances parotid gland tumors, after a latency period varying from 2½ to 3½ months(8). After several consecutive passages through newborn hosts, an attempt was made to determine whether this highly potent passage agent might now also induce leukemia when inoculated into suckling mice older than 16 hours. In contrast to previous experience, C3H mice 1 to 14 days old were found highly susceptible to inoculation of the leukemic filtrates; young adult C3H mice were also susceptible, though to a considerably lesser extent.

Methods. Preparation of leukemic extracts. C3H mice which developed advanced leukemia at 2½ to 3½ months of age as a result of inoculation, when newborn, with filtrates prepared from the 8th, 9th, or 10th cell-free passages, were used as donors. Parts of liver, spleen, mediastinal and mesenteric tumors, and peripheral lymph nodes, were removed aseptically, weighed, ground in a mortar by hand with chilled, sterile physiological saline added to obtain 20%, and in a few instances 10% concentration. Following centrifugation at 0°C, first at 3000 rpm (1400 x g) for 15 minutes, then at 9500 rpm (7000 x g) for 5 minutes, the final supernate (10 to 12 ml) was mixed with 0.5 ml of 1:2000 dilution of fresh broth culture of *E. coli*, and passed through Selas porosity 02 porcelain filter candles under vacuum pressure(10). In each instance the filtrate was checked and found to be sterile as evidenced by inoculation of tryptose broth media, thus indicating that the filter candle retained *E. coli*. The filtered leukemic extracts were immediately placed in sterile glass tubes immersed in larger containers filled with ice cubes. Kept at 0°C, most of the extracts were used within 24 hours, none later than after 48 hours. *Test animals.* All mice used for inoculation, except when otherwise stated, were of the C3H, or foster nursed C3H(f) lines, both of Bittner substrain(9). The sexes were separated at weaning time. Those mice that died when less than 6 weeks old, were not included in the tabulation; this concerns particularly the mortality occurring among newborn mice within hours after inoculation. The incidence of spontaneous leukemia, or lymphosarcomas, in untreated mice of our colonies of C3H or C3H(f) mice does not exceed 0.5%, and occurs in mice usually over 15 months of age.

Results. Inoculation of filtrates into newborn mice less than 16 hours old. Filtered extracts prepared from passage 8th, 9th and 10th donors, were inoculated into newborn, less than 16 hours old C3H mice (passage 8th refers to inoculations of extracts prepared from passage 7th donors, passage 9th to inoculations from passage 8th donors, etc.) Of 310 mice, 175 (56%) developed leukemia, and 24 (8%) developed parotid gland carcinomas, at 3.9 months average age. Centrifuged (7000 x g) extracts were more potent than filtrates, 40 mice out of 44 inoculated

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TABLE I. Results of Inoculation of Centrifuged or Filtered Leukemic Passage Extracts into Newborn, Less than 16-Hr-Old C3H Mice.

Passage No.	Extract inoc.*	No. mice inoc.*	No. mice dev. leuk.	Leuk. inc., %	No. mice dev. parot tum	Parot tum inc., %	Total dev. leuk. or parot tum	Total leuk. & parot tum inc., %	Avg age leuk. or parot tum dev., mo
8	Fil	64	23	36	15	23	38	59	4
	Ctr	32	28	88	1	3	29	91	3.5
9†	Fil	120	73	61	1	1	74	62	4
	Ctr	12	12	100					3.5
10†	Fil	126	79	63	8	6	87	69	3.6
Total	Fil	310	175‡	56	24§	8	199	64	3.9
	Ctr	44	40	91	1	2	41	93	3.5

* Fil = Selas 02 filtrate. Ctr = 7000 x g supernate. All inoculations subcut. Those mice that died when less than 6 wk old are not included in tabulation.

† These experiments are still in progress; avg age of surviving mice, still in good health, is 7 and 5 mo for passage 9th and 10th respectively.

‡ 2 mice developed leukemia and parotid tumors.

§ 2 " " parotid tumors and medullary adrenal tumors.

developing leukemia (91%), and 1 parotid gland tumors (2%) at 3.5 months average age (Table I).

Inoculation of filtrates into suckling mice 16 hours to 21 days old. When suckling mice more than 16 hours old, and up to 14 days of age were inoculated with the filtered leukemic extracts (Table II), 243 out of 304 (80%) developed leukemia at average ages varying from 3.3 to 3.7 months, some as early as 2 months after inoculation. None developed parotid tumors. The incidence of leukemia was higher in mice inoculated intraperitoneally (89%) as compared with those inoculated subcutaneously (69%). Of 28 mice inoculated when more than 14, and up to 21 days of age, only 9 (32%) developed leukemia; this incidence may, however, increase since some of the mice in this group are now only 4 months old.

Inoculation of filtrates into adult mice. The leukemic filtrates were inoculated intraperitoneally or intracranially into 122 young adult C3H mice (average age at inoculation 51 days) and, as a result, 45 of them (37%) developed leukemia at an average age of 8.8 months. None developed parotid tumors (Table III).

Transplantation of leukemic cells from passage 8th, 9th and 10th donors. In 10 experiments, leukemic cell suspensions of 20% concentration were prepared from 8th, 9th and 10th passage donors (in which leukemia resulted from inoculation of filtrates); these cell suspensions were then injected intraperitoneally (0.25 to 0.5 ml) into young adult (2 months old) C3H and Ak mice. All 17 inoculated C3H mice, and 16 of 18 inoculated Ak mice developed leukemia after average latency of 17 and 61 days respectively.

Inoculation of leukemic filtrates into newborn C3H mice of Andervont subline. Young, adult C3H males and females were recently obtained from Dr. H. B. Andervont, Nat. Cancer Inst. These mice were mated in our laboratory, and their newborn offspring were inoculated at average age of 10 hours with leukemic filtrates (Selas 02) prepared from passage 8th, 9th and 10th donors. Of 33 mice only 4 (12%) developed leukemia at 2½, 3, 4, and 4½ months respectively; none developed parotid tumors. In simultaneous experiments, the same filtrates were inoculated into 25 less than 16 hours old C3H mice of the Bittner substrain, and 14 (56%) developed leukemia at average age of 2.8 months. Although not yet completed, these results are essentially similar to those previously reported(9).

Discussion. When in previous experiments cell-free extracts prepared from spontaneous Ak leukemia were inoculated into suckling C3H mice 2 to 12 days old, some of them developed leukemia, but not before 14.5 months of age(2). The potency of the leukemic agent increased through serial cell-free pas-

TABLE II. Results of Inoculation of Filtered Leukemic Passage Extracts* into Suckling C3H Mice 16 Hours to 21 Days Old. None developed parotid tumors.

Age at inoc., days	Route of inoc.‡	No. of mice inoc.§	No. of mice dev. leuk.	Leuk. inc., %	Avg age leuk. dev., mo
16 hr <1†	Subcut.	34	21	62	
	Intraper.	3	3	100	
		37	24	65	3.3
1 < 2	Subcut.	54	40	74	
	Intraper.	13	13	100	
		67	53	79	3.6
2 < 4	Subcut.	38	26	68	
	Intraper.	34	31	91	
		72	57	79	3.3
4 < 7	Subcut.	8	5	62	
	Intraper.	56	49	87	
		64	54	84	3.4
7 <10	Subcut.	3	3	100	
	Intraper.	26	25	96	
		29	28	97	3.6
10 <14	Intraper.	35	27	77	3.7
Total: 16 hr <14 days	Subcut.	137	95	69	
	Intraper.	167	148	89	
		304	243	80	
14 <21	Intraper.	28	9	32	

* All extracts were Selas 02 filtrates prepared from passage 8th, 9th and 10th.
† More than 16 hr, and up to 1 day old.
‡ Subcut. = subcutaneous; Intraper. = intraperitoneal.
§ Those mice that died when less than 6 wk old are not included in tabulation.

sage; the agent now induces leukemia after a latency of 2½ to 4 months when inoculated into suckling mice 1 to 14 days old. Additional experiments are needed, however, to determine whether at least some of the more potent extracts prepared from Ak mice with spontaneous leukemia, may be more active than previously suspected, when inoculated into suckling C3H mice less than 14 days old.

Serial, cell-free passage of the leukemic agent might have increased its potency for the Bittner substrain of the C3H line, increasing incidence of induced leukemia, shortening latency time, and extending age range of susceptibility of the recipient hosts. The ability, however, of the agent to infect recipient hosts of certain genetically distinct inbred lines, did not change materially. A subline of the C3H strain, maintained in Dr. Andervont's laboratory at the National Cancer Institute(9), remained relatively resistant to the passage A leukemic strain.

In previous experiments, when leukemia was induced in C3H mice with Ak leukemic filtrates, leukemic cell suspensions prepared from C3H donors could be readily transplanted to adult C3H hosts, but only rarely to adult mice of the Ak line(11,7,3). It is quite interesting, therefore, that C3H leukemia induced with the potent passage filtrates, could now be transplanted by cell-graft to adult mice of both, C3H and Ak inbred lines(4); the latency period, however, was considerably longer in Ak mice inoculated with the C3H leukemic cells (61 days) as compared with C3H recipients (17 days).

The fact that inoculation of passage A leukemic filtrates into C3H mice more than 16 hours old induced leukemia, but not parotid gland tumors, was puzzling. Of 304 mice inoculated when more than 16 hours, but less than 14 days old, 80% developed leukemia, none parotid tumors (Table II). The same filtrates inoculated into 310 mice less than 16 hours old induced either leukemia (56%) or

TABLE III. Results of Inoculation of Leukemic Passage Filtrates* into Adult C3H Mice.†

Route of inoc.‡	No. of mice inoc.	No. of mice dev. leuk.	Leuk. inc., %	No. of mice dev. parot tum	Avg age leuk. dev., mo	Avg latency, mo
Intraper.	94	35	37	0		
Intracran.	28	10	36	0		
Total	122§	45	37	0	8.8	7

* All extracts were Selas 02 filtrates. Each filtrate was prepared from a different donor.

† 86 were C3H or C3H(f) males; 28 were C3H and 8 C3H(f) females.

‡ Intraper. = intraperitoneal (0.5 to 1.0 ml). Intracran. = intracranial (0.1 to 0.15 ml). Avg age at inoculation, 51 days.

§ 38 mice still alive and well at 12.5 mo avg age. 39 died with no signs of leukemia at 14 mo avg age.

parotid tumors (8%), occasionally both (Table I). Similarly, leukemia (37%) but not parotid tumors developed following inoculation of the filtrates into adult C3H mice (Table III). The striking difference in susceptibility to the induction of parotid tumors, depending on the age of the recipient host at the time of inoculation, may be consistent with the assumption previously expressed that the leukemic agent, and that causing parotid gland tumors, are distinct, though possibly related(10,7).

Previous attempts to transmit leukemia into adult C3H mice with filtrates prepared from initial passages, were seldom successful (8). It was surprising, therefore, to find that filtrates prepared from the more recent passages induced leukemia in 45 out of 122 C3H mice inoculated when approximately 6 to 8 weeks old. Adult hosts were, however, less susceptible than 1 to 14 days old suckling mice; furthermore, the disease appeared late, at 8.8 months average age (Table III). Our passage A leukemic agent is therefore considerably less pathogenic for adult hosts than that developed from Ehrlich ascites carcinoma in Swiss mice by Friend(12).

It is of interest that centrifuged extracts were more potent than filtrates (Table I), and that the intraperitoneal route of inoculation was more effective than subcutaneous (Table II).

The fact that passage A leukemic filtrates were found to be highly active when inoculated into few-days-old C3H mice is of practical value. It may no longer be necessary to prepare for inoculation extracts only when female mice in advanced pregnancy are available, and then check such mice every few hours for litters, in order to inoculate newborn mice possibly within hours after birth. Several litters can now be accumulated in preparing an experiment, and the extract then made and injected at once into several groups of susceptible suckling mice. Furthermore, mortality resulting from inoculating more than 1-day-old suckling mice is practically nil, even when the more sensitive intraperitoneal route is used, whereas mortality is often very high when newborn mice, less than 16 hours old, are inoculated. Further observations are needed to determine whether suckling mice less than 14 days old are uniformly susceptible to inoculation of leukemic filtrates, and whether there are differences in susceptibility between newborn (less than 16 hours old) mice, and those 2, 5 or 10 days old. It is quite possible that suckling C3H mice 2 to 10, and perhaps even up to 14, days old, may be more susceptible to the leukemogenic action of the passage filtrates than newborn, less than 16 hours old mice.

In studies on chicken leukosis(13-16) and also on Rous sarcoma(17), the observation has long been made that among the susceptible recipient hosts, very young animals were more sensitive to inoculation of the cell-free extracts. There appears to be an optimum age for inoculation of the filtrates, which is different for different forms of chicken leukosis (13-15) and also for different strains of chickens in the case of Rous sarcoma(17).

Summary. 1. After 7 consecutive passages through newborn hosts, passage A leukemic filtrates could be inoculated successfully into suckling C3H mice 1 to 14 days old, inducing leukemia, but not parotid tumors, in 62% to 100% of the inoculated mice at 3.3 to 3.7

months average age. 2. The incidence of leukemia was higher in mice inoculated intraperitoneally (89%) than in those injected subcutaneously (69%). 3. Of 122 young, adult C3H mice inoculated intraperitoneally, or intracranially with leukemic filtrates, 37% developed leukemia (none parotid tumors) at 8.8 months average age. 4. Of 310 newborn C3H mice inoculated when less than 16 hours old with the same leukemic filtrates, 64% developed either leukemia (56%), or parotid tumors (8%), at 3.8 months average age. Two mice in this group developed both leukemia and parotid tumors.

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Plasma Heparin Levels in Man Following Intravenous Fat Emulsions. (23723)

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Clearing of alimentary lipemia(1) and decrease in serum low density lipoproteins(2) following injection of heparin have raised the question of the possible physiologic role of heparin in the blood transport phase of fat metabolism. The concept has been strengthened by identification(3) of the tissue enzyme, lipoprotein lipase, of which heparin is probably a component(4), and demonstration (5) of a similar lipolytic enzyme in the plasma of some normal individuals. The inverse correlation found between human plasma heparin levels and serum low density lipoproteins(6) added further evidence. This report will present data showing an increase in the level of plasma anticoagulant substances (heparins) following administration of intravenous fat emulsions in man, indicating that fat intake is a stimulus for release of heparin into the bloodstream.

In preliminary experiments(7) increase in plasma metachromatic substances was found in 6 of 10 individuals following intravenous injection of cottonseed oil emulsion. The method used for extraction and separation of endogenous circulating heparin in the earlier study was a modification of the technic suggested by Nilsson and Wenckert(8) in which heparin is adsorbed on tricalcium phosphate gel, then eluted with citrate, and the octylamine-brucine method of Jaques(9) applied to the eluate. However, the use of this method of heparin extraction was not continued since other metachromatic substances, such as chondroitin sulphate, may be present in blood. Moreover, it was found that the octylamine

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