TABLE I. Assay of MSH Preparations from 10 Placentas from 10 Patients.

U*/mg	U*/mg
3.60×10^2	2.65×10^2
2.75 "	4.80 "
1.30 "	2.70 "
4.00 "	3.20 "
3.10 "	2.40 "

* Unit of activity is defined as degree of darkening, measured with photoelectric reflection meter, produced by .04 μg of lyophilized water extract of beef pituitary powder on isolated frog skin(10).

about 7.9 U/g)(6). This makes a hypothetical placenta: whole blood gradient of more than 30,000:1. This material seems present in concentrations greater than can be accounted for by its being derived from placental blood.

This study does not differentiate between placenta as site of storage or site of production of an MSH-like substance. Experiments attempting to answer this question are projected.

It is known that melanocyte stimulating activity is intrinsic to corticotropins. In this study it was noted that after alkalinization and heating, which destroys much of the corticotrophic activity(9), the melanocyte stimulating activity was enhanced 20 to 30 times. In addition, the darkening was qualitatively somewhat different, with a prolonged and slower darkening caused by the alkali treated material.

Conjecture regarding the biological significance of placental MSH may be summarized as follows: The rapid decline of MSH values in blood and in urine soon after delivery (6) might be due to loss of the placenta containing large quantities of this substance. Whether the formation and rapid post partum loss of much of the increased dermal and mucosal pigmentation commonly noted during pregnancy (chloasma) is also related to placental MSH requires further study.

Conclusion. An MSH-like substance was present in considerable concentration in human placenta.

I am grateful to Dr. R. W. Wissler for encouragement and support, to Dr. R. Stoughton for helping initiate this work. Research Laboratories of Armour & Co. were extremely generous in providing material. The following have kindly given me criticism and suggestions: Dr. Z. Hruban, Dr. E. J. Plotz, Dr. J. Fisher and Mr. L. Frazier.

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Received January 8, 1959. P.S.E.B.M., 1959, v100.

Prevention of Viral Lymphoid Leukemia of Mice by Thymectomy.*† (24717)

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Most animals of the high leukemia mouse strains Ak and C58 die of spontaneous leukemia, and all transmit the genetic liability for leukemia to their offspring, whether or not the parents die of leukemia. These genetically determined leukemias originate in the thymus

* Supported by grants of Nat. Inst. of Health and Am. Cancer Soc.

† We acknowledge the skillful technical assistance of Mrs. Sah Sook Cho.

for the most part, and are preventable by thymectomy. Virus-like agents capable of inducing leukemia have been isolated from these high leukemia strains. An experiment was set up to determine whether thymectomy will prevent induction of leukemia by a virus originally derived from Ak leukemia tissue.

Material and methods. Virus. The passage virus, "Agent A" of Gross(1) was used. Filtrates produce about 50% leukemias in C3H/f/G recipients in less than 6 months after injection in the newborn period, and extracts produce an even higher percentage with a shorter latent period(1). Our material was derived from 2 animals (one each from passages 10 and 11) sent to us by Dr. L. Gross in November, 1957. A filtrate was prepared from the pooled leukemic tissues of these 2 mice and frozen at –70°C. Vials containing 1 cc aliquots were thawed as needed and injected into 112 C3H/f/B/G/L‡ (hereafter designated Z) baby mice less than 7 days of age (passage 12). Two leukemic mice from passage 12, one injected <40 hours, the other <8 hours of age, were sacrificed at 77 and 111 days respectively. A filtrate was prepared from each animal (passage 13) and stored in sealed ampoules at –70°C until used. *Filtrate preparation.* Lymph nodes, a piece of liver (excluding gall bladder), spleen and thymic tumor of leukemic mice were harvested aseptically, ground in a chilled mortar, diluted to a 15-20% suspension with chilled normal saline and centrifuged at 0°C for 15 minutes at 1500 g. The fatty scum occasionally found on the surface was removed with a sterile swab, and the supernatant fluid centrifuged at 6,900 g for 10 minutes at 0°C. The second supernatant fluid to which *E. coli* was added was passed through a chilled Selas 02 filter previously moistened with chilled normal saline. Cultures of material before filtration had abundant growth, while the filtrate was sterile. The filtrate was divided into 1 cc aliquots in small vials, sealed and stored at –70°C, after first chilling for half an hour in the freezing compartment of an ice box. Vials were thawed as needed during 2 months after preparation. *Treatment of mice.* A total of 187 Z mice were injected i.p. with 0.025 to 0.07 cc at less than 7 days of age with these 2 filtrates (passage 13). Of these, 99 survived 60 days. Since there was no marked difference in infectivity of these 2 filtrates the data are combined. Alternate litters were set aside as controls. At about the age of 4-6 weeks, alternate animals in each injected litter were *thymectomized* under ether-nembutal anaesthesia. Fourteen alternate uninjected controls were also thymectomized. Intercurrent infections in all animals were treated by the addition of veterinary oxytetracycline or chloramphenicol to the drinking water, and in some cases by injection of a streptomycin-penicillin mixture. Males and females were caged together. Babies born to virus-injected animals were destroyed after birth; those from uninjected controls were raised. All animals that died were autopsied and tissues of many leukemic and non-leukemic mice were examined histologically. At 7 to 9 months of age all surviving animals were sacrificed, autopsied, and many normal appearing mice studied histologically. In addition, bioassays of normal appearing thymuses and lymphoid tissues of virus injected and uninjected control animals, selected at random, were performed for presence of leukemic cells and possible persistence of virus. The bioassay (grafting lymphoid tissues on histocompatible normal young adults) is in progress.

Results. There were 25 leukemias among 50 virus-injected intact mice but only 1 in 49 virus-injected thymectomized mice, and none in uninjected controls. Fig. 1 shows total cumulative leukemia mortality in virus injected animals. All but one of the 25 leukemias found in virus-injected intact animals had a thymic tumor, most of these with gross enlargement of the spleen and involvement of lymph nodes.

No other tumors or evidence of "polyoma" virus(2) activity were found in spite of search by both gross and microscopic examination of salivary glands, thymus, kidney and other organs.

‡ This sybmol is that recommended by G. Snell, indicating C3H mice, foster nursed, originally obtained from Bittner and carried for several generations by Gross and his subline by Levinthal.

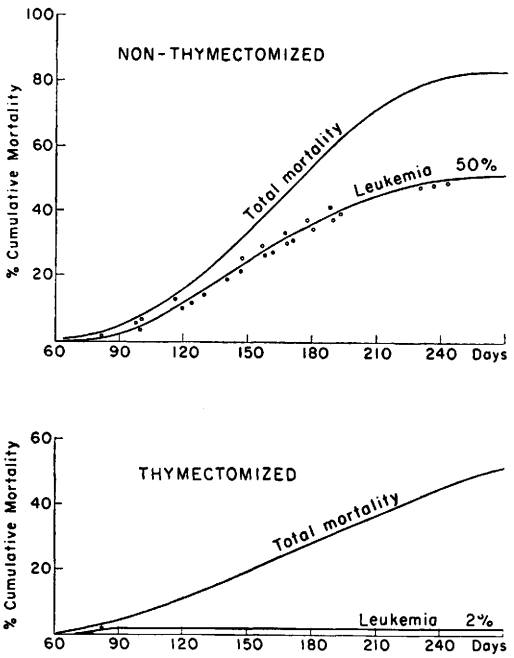

FIG. 1. Cumulative total and leukemia mortality (% of total group) in thymectomized and non-thymectomized virus infected Z mice. On leukemic line each dot represents a leukemic mouse.

Table I gives the cumulative mortality and leukemia incidence in all experimental and control groups in this study.

Passage 13 virus caused somewhat fewer leukemias than passage 12, but this can be explained mainly by intercurrent pneumonia of an interstitial non-bacterial type. In general, the difference in mortality among the infected groups was due to leukemia, the non-leukemic mortality being comparable in all 4 groups (Table I).

In both passages, there was a slightly higher incidence of leukemia in females (average 59%) than in males (54%) and a slightly shorter latency period (Table II), regardless of age at time of injection.

These findings parallel the observations on spontaneous leukemia in Ak mice(6) in which the frequency is greater in females and is markedly reduced by intercurrent pneumonia. Reduction of leukemia frequency by pneumonia can be attributed to partial thymic involution due to stress, mediated by the adrenal cortex. This has been duplicated by direct administration of cortisone.

Fig. 2 shows the data on infected non-thymectomized mice and controls in both passages indicating death from leukemia and other causes, distinguishing between animals found dead and sacrificed at termination of experiment. On basis of available information termination of such experiments at 240-270 days, as done here, is permissible for first approximation. Earlier studies have indicated that the leukemias occurring at later age are not thymic and are not influenced by thymectomy(6).

Discussion. Agents capable of inducing leukemia in genetically susceptible strains of mice include whole body irradiation, carcinogens such as methycholanthrene, and viruses

TABLE I. Cumulative Mortality from Leukemia and Other Causes.

	Mice	No. in group†	Age, days: 61-90 L*	61-90 T*	91-120 L	91-120 T	121-150 L	121-150 T	151-180 L	151-180 T	181-210 L	181-210 T	211-240 L	211-240 T	241-270 L	241-270 T
	Passage 12															
I	Virus infected non-thymectomized	77	1	1	8	10	23	28	33	39	42	49	46	56	46	57
II	Non-infected	78	0	1	0	3	0	3	0	8	0	11	0	21	0	25
	Passage 13															
I	Virus infected															
	(a) Thymectomized	49	1	2	1	7	1	9	1	14	1	17	1	23	1	25
	(b) Non-thymectomized	50	1	3	6	9	11	18	19	29	22	37	24	41	25	42
II	Normal, uninj.															
	(a) Thymectomized	14	0	2	0	3	0	5	0	6	0	6	0	6	0	8
	(b) Non-thymectomized	27	0	2	0	2	0	6	0	7	0	10	0	10	0	12

* L = leukemia mortality; T = total mortality.
† Discounting those dying younger than 60 days of age.

TABLE II. Viral Infectivity in Relation to Sex.

	Males			Females		
	No. L/No. inj.	%	Latency, days (avg)	No. L/No. inj.	%	Latency, days (avg)
Passage 12	25/43	58	168	21/34	62	148
" 13	12/26	46	169	13/24	54	148
Combined	37/69	54	168	34/58	59	148

(1,3,4,5). The virus of Gross and the non-viral agents usually mediate their effect through the thymus. Thymectomy reduces incidence of spontaneous leukemia in the high leukemia strains Ak(6), and C58(7) in which leukemia has been related to both genetics and virus. Similarly, thymectomy prevents development of thymic lymphomas following whole body irradiation(8) or administration of methycholanthrene(9). Thymic homografts in thymectomized mice of low leukemia strains reestablish the increased leukemia incidence which follows administration of methycholanthrene(9) or x-rays(10).

The present experiment indicates that the virus derived from Ak material requires the thymus to cause leukemia, within the time limit of our study.

By what mechanism thymectomy prevents leukemia is still to be established. Numerous workers noted that the thymus has a lymphocytosis stimulating factor (LSF). As early as 1909, Maximow(11) observed that the thymic epithelial anlage attracts lymphocytes,

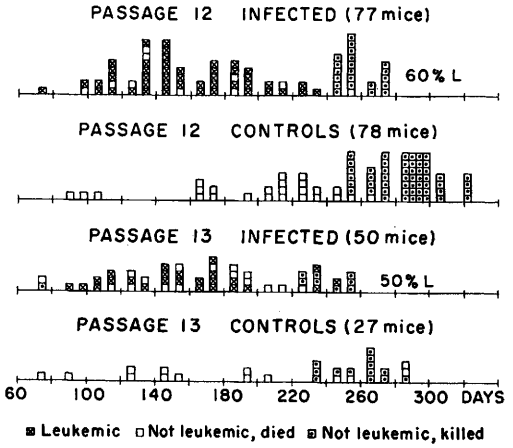

FIG. 2. Histogram of infected (not thymectomized) mice and controls in passages 12 and 13 indicating death from leukemia and other causes and distinguishing between animals found dead and sacrificed at termination of experiment.

and stimulates their proliferation within the organ. This work was carried further by Jolly(12), Gregoire(13), Law(14), Kaplan (15), and Metcalf(16). Gregoire(13) noted that implants or extracts of thymic epithelial tissue (heterologous thymus depleted of thymic lymphocytes by irradiation, leaving the radio-resistant epithelial stroma) stimulated lymphopoietic activity in regional lymph nodes while implants of lymph nodes or musle fragments were ineffective. Metcalf(16) correlated the high level of circulating lymphocytes in the high leukemia strains Ak and C58 with a greater blood level of LSF during their preleukemic period than in low leukemia strains. But this does not explain why the leukemic lymphocytes behave as autonomous cells; excessive stimulation should cause lymphocytosis, *i.e.*, an increase of normal but not autonomous cells.

The question is, therefore, what brings about autonomy? The apparent autonomy of viral leukemia is indicated by its transplantability. Is autonomy due to mutation of thymic lymphocytes under the influence of virus, x-rays or carcinogens? Is this influence localized in the thymus because of a genetic affinity of this organ to virus, or because of LSF, which is produced by the thymic epithelial reticulum and is in proximity with thymic lymphocytes? Is a specific growth factor required for leukemic transformation of lymphocytes? The latter hypothesis is suggested by the work of Law(14) indicating that the lymphocytes of some lymphomas arising in thymic grafts originate in the host.

If direct cancerization of thymic lymphocytes by virus is postulated (without the aid of LSF), one must assume that thymic lymphocytes are different from other lymphocytes, and that thymectomy achieves physical removal of lymphocytes most susceptible to malignant transformation. If a 2-stage process

is postulated (virus and LSF), it may be possible to induce lymphoma by virus in lymph nodes by providing adequate concentration of LSF. A third possibility is that the thymic epithelial reticulum is an especially good culture medium for the virus, and that a high concentration of virus is necessary for leukemia induction.

Data presented by Gross suggest the assumption that the virus causing lymphoma in Ak mice is different from that causing multiple tumors. The observations of the present studies in conjunction with those of Buffett *et al.*(17), carried out simultaneously in this laboratory, strongly support this assumption. A critical experiment in which these 2 different materials with viral activity were injected in the same type of host, now in progress, supports this view.

Earlier experiments(6) have shown that transplantation of leukemic cells is not influenced by absence of thymus. Therefore, prevention of leukemias in thymectomized animals demonstrates that leukemic cells did not pass through the filters.

Summary and conclusions. 1. Ninety-nine Z mice were injected with Gross "Agent A" (Selas filtrates). Leukemia was induced in 1 of 49 animals thymectomized at 4-6 weeks of age, and in 25 of 50 alternate non-thymectomized mice. No leukemias occurred in 41 uninjected controls. The experiments were terminated when the animals were 7-9 months of age. 2. Incidence of leukemia induced by virus was greater and the latent period shorter in females than in males. 3. Spontaneous Ak leukemia, from which this agent is derived, behaves similarly to viral leukemia with respect to relaton of sex to incidence and latency period. However, spontaneous leukemia occurs at a later age than this viral leukemia. 4. The thymus is the fulcrum of both lymphoma induction by this virus and of its spontaneous development in Ak mice; its presence is essential for development of this type of leukemia.§

§ Since this paper was submitted, we have learned of similar results in Ak mice obtained by Dr. J. F. A. P. Miller of Chester Beatty Research Institute, submitted to *Nature*.

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