renal function although the effect of ACTH is more pronounced than that of cortisone.

The uropepsin excretion in the urine reflects the peptic activity of the stomach. It is derived from the secretion of pepsinogen directly into the blood stream by the peptic cells of the stomach. Pepsinogen is then transported to the kidneys and is excreted in the urine as uropepsin(8). That uropepsin has its origin in the stomach is evidenced by the disappearance of the enzyme following total gastrectomy and by its absence in the urine of patients with pernicious anemia(9).

9. Gray, S. J., Spiro, H. M., and Reifenstein, R. W., *Proc. First Clinical ACTH Conference,* Blakiston, 1950, Phil., 177; Spiro, H. M., Reifenstein, R. W., and Gray, S. J., *J. Lab. and Clin. Med.,* 1950, v35, 899.

Summary. ACTH administered intramuscularly to normal subjects in doses of 100-160 mg daily for periods of 3-4 weeks produced a marked increase in the basal gastric secretion of hydrochloric acid and pepsin associated with an equally significant rise in the urinary uropepsin excretion. In every instance these values were increased to the levels usually observed in patients with active peptic ulcer. These studies indicate that an endocrine relationship exists between the stomach and the adrenal gland and that a hormonal phase of gastric secretion may be mediated by the adrenal corticoids. The pathway by which chronic emotional and physical stress may affect the stomach by a hypothalamus-pituitary-adrenal-gastric pathway is discussed.

Received October 3, 1951. P.S.E.B.M., 1951, v78.

Pathogenic Properties, and "Vertical"* Transmission of the Mouse Leukemia Agent.† (19068)

LUDWIK GROSS.

From the Cancer Research Unit, Veterans Administration Hospital, Bronx, New York.

It has been recently observed in this laboratory that "spontaneous" leukemia could be prompted to develop in middle aged mice of a foster nursed C3H subline, designated by the symbol C3H(f), and known to be essentially free from this disease, by inoculating such animals within the first 12 hours after birth with centrifugated extracts prepared from leukemic mice of the Ak line, or with normal Ak embryo cell suspensions. Generalized leukemia developed in some of the inoculated animals after they had reached 8 to 11 months of age(2).

This report deals with the inoculations of the centrifugated leukemic extracts into infant mice 12 hours to 6 days old, with the results of preliminary experiments on the filtration of the leukemic agent, and with the transmission of the leukemic agent from parents to their offspring.

Materials and methods. Centrifugated leukemic extracts. Mice of the Ak line that developed leukemia either spontaneously, or as a result of inoculation with a transplanted strain of Ak leukemia(3), were used as donors

* Vertical transmission designates the passage of a parasitic agent from one generation to another(1), such as rickettsia through eggs in ticks, mosaic virus through seeds in plants, or mammary carcinoma virus through milk in mice. In contrast, horizontal transmission occurs from one individual to another within the same generation, as in smallpox, typhoid fever, or malaria, through contact, carriers, food, or insects, respectively.

† Aided, in part, by a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc., through Columbia University.

Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the results of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Gross, L., *Surg. Gynec. and Obst.,* 1949, v88, 295.

2. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 27.

TABLE I. "Spontaneous" leukemia developing in middle aged mice of the C3H(f) line, following subcutaneous inoculation,* in early infancy, with Ak leukemic, *centrifugated* extracts.

Exp. no.	Age at inoc., days	Sex	No of mice inoc.	No. of mice devel. leuk.	Age when leuk. appeared, mo	No. of mice died without signs of leuk.	Age of mice dying not from leuk., mo	No. of mice in good health at this time	Age of mice in good health at this time, mo
1100	<½	F	1	1	9				
		M	4	3	9,11,17	1	17		
1101	<½	F	1	1	9				
		M	3	2	8,17	1	19		
1106	<½	F	1	1	18				
		M	4	4	11,11,14,18				
1261-A	<½	M	2	1	11	0		1	14
1109-A	<1	F	1	0				1	19
		M	3	2	18,19	1	14		
1361-A	<1†	F	2	2	4,9			0	
		M	2	1	9	1	5	0	
1361-B	<1†	F	1	1	9				
		M	1	1	7				
1361-C	<1†	F	4	4	4,5,8,9				
		M	1	1	8				
1373	<1†	F	5	4	6,9,9,9½			1	9
1261-B	2	M	2	2	14½,15½				
1100-B	3	F	2	0		1	14	1	19
		M	3	0		1	15	2	19
1149	4	F	4	1		0		3	19
		M	1	0				1	19
1109-B	5	F	4	4	18,18,18,19				
		M	1	1	18				
1109-C	6	F	2	2	18,20				
		M	3	2	18,18			1	20
	Total		58	41		6		11	

* 0.1 cc of centrifugated leukemic extract of 20% conc.

† These mice were first inoculated (0.1 cc each) when they were less than 1 day old; the remaining leukemic extract was then placed in refrigerator at 0°C, 24 hr; the same infant mice were then reinoculated with the extract (0.1 cc each); a third inoculation of these mice with the same refrigerated extract (0.1 cc each) was then made after additional 48 hr.

of the leukemic organs. Liver, spleen, mesenteric tumor, and peripheral lymph glands of such mice were cut, and ground, physiological solution of sodium chloride being added to obtain cell suspensions of 20% concentration; these cell suspensions were then centrifugated at 3000 RPM (1400 x g) for 15 minutes at 0°C in an International PR-1 Centrifuge; the supernatant was removed and then again centrifugated for 15 minutes at 3000 RPM. The second supernatant was then immediately used for inoculation. A tuberculin syringe and a 27 gauge needle were used for the injection of the suckling infant mice. *Animals.* Mice of a foster nursed C3H subline (C3H(f)) or, in certain experiments mice of the C3H line, were used for inoculations. The incidence of spontaneous leukemia in our colonies of these mice has been less than 0.07%, only 3 cases of spontaneous leukemia having occurred among the more than 5,000 mice of both sexes during the past 6 years (2), and only 1 case of spontaneous leukemia among more that 1500 males and females of the foster nursed C3H(f) subline.

Experimental. Inoculation of centrifugated leukemic extracts. In our preceding report (2), of the 14 C3H(f) mice inoculated when less than 12 hours old with centrifugated Ak leukemic extracts, 7 developed leukemia at 8 to 11 months of age; later on, however, 5 additional mice of this group also died from leukemia; thus, in these 3 experiments (Exp. No. 1100, 1101 and 1106, Table I) a total of 12 out of 14 mice, inoculated when less than 12 hours old with leukemic extracts, developed "spontaneous" leukemia. The results of experiments on the inoculation of C3H(f)

TABLE II. Results of subcutaneous inoculation of *filtered* Ak leukemic* extracts into suckling infant mice of the C3H(f) line.

Exp. No.	Age at inoc.	Sex	No. of mice inoc.	No. of mice develop. leuk.	Age when leuk. appeared, mo	No. of mice died without signs of leuk.	Age of mice dying not from leuk. mo	No. of mice in good health now	Age of mice in good health now, mo
1533	3 hr	F	2	0				2	7
		M	2	1	6½			1	7
1432	< 7	F	4	2	8½,9			2	9
		M	3	0				3	9
1550	<12	F	2	0				2	7
		M	3	1	7			2	7
1420	<12	M	3	1	6½			2	9
1447	<12	F	4	0				4	9
		M	2	2	8½,9				
764-A	8 days	F	2	1	23	1	14		
		M	1	0		1	14		
764-B	8	F	2	1	27	1	14		
		M	2	0		2	16,18		
	Total		32	9		5		18	

* In Exp. 1420, 1432, 1533 and 1550, the suckling mice were inoculated with a filtered extract prepared from spontaneous Ak leukemia. In exp. 1447, 764-A, and 764-B, the filtered extract was prepared from a transplanted[2] Ak leukemia. In exp. 764-A and 764-B the dose inj. was 0.25 cc for each mouse. In all other experiments infant mice were inj. with 0.1 cc each.

infant mice, when 12 hours to 6 days old, with the centrifugated extracts of Ak mouse leukemia, are summarized in Table I. It is evident from these experiments that when infant, suckling mice of the C3H(f) line were inoculated when less than 1 day old, most of them developed "spontaneous" leukemia after they had reached 7 to 11 months of age; in a few instances, some of these mice that were first inoculated when 1 day old, and were again twice reinoculated to increase the total dose injected, developed leukemia even before they had reached 6 months of age (Exp. 1361 A, B, C, and 1373, Table I). When infant mice 3 to 6 days old were inoculated with the centrifugated leukemic extracts, they developed "spontaneous" leukemia only after they have reached 18 months of age, or not at all (Exp. 1100-B, 1149, 1109-B and 1109-C, Table I).

Experiments are now in progress to determine whether C3H(f) mice that had been inoculated with the centrifugated Ak leukemic extracts at 7 to 21 days of age, will eventually also develop leukemia. No leukemia has yet developed in these groups of mice, although more than 18 months have elapsed since some of them were inoculated.

Inoculation of filtered leukemic extracts. The filtered leukemic extracts were prepared in the following manner: A leukemic cell suspension of 20% concentration, freshly prepared in the usual manner(2), from either spontaneous or transplanted(3) Ak leukemia, was centrifugated at 3,000 RPM for 10 minutes, and the supernatant was then passed through a Seitz (S-1 pad) filter, using a water aspirator, under a vacuum pressure of approximately 20 mm of mercury. The filters, with the mounted pads, had been autoclaved, prior to their use, at 15 lbs, 115°C, for 1½ to 2 hours. Immediately after the filtration, the filters were tested with, and were in all instances found to be impervious to, *E. coli*. The suckling infant mice of the C3H(f) line were then promptly inoculated subcutaneously with the filtered extracts. The results of 7 individual experiments on the injection of the filtered leukemic extracts into suckling C3H(f) mice are summarized in Table II. Of the 9 mice that developed leukemia among the 32 inoculated with the leukemic filtrates, none had any evidence of a local growth at the site of the initial subcutaneous inoculation. In 5 experiments, the filtrates were injected into suckling mice less than 12 hours old. Of the 25 mice injected, 7 developed "spontaneous" leukemia at 6½ to 9 months of age; the remaining 18 mice, however, are still

only 7 to 9 months old at this time, and it is possible that some of them may later also develop leukemia. In Exp. 764 A and B, older (8 days) C3H(f) mice were injected, because at the time when this experiment was carried out (September 15, 1948) we did not know that the susceptibility of the C3H(f) mice to inoculation with the Ak leukemic agent decreases so rapidly after the first day of life. Of the 7 inoculated mice, 2 developed leukemia at 23 and 27 months of age respectively (Table II). This delayed development of "spontaneous" leukemia in these mice is similar to that observed in those groups (Table I), where older infant mice were inoculated with centrifugated leukemic extracts.

Transmission of the leukemic agent from parents to offspring. Exp. LV-7. On December 1, 1949, 4 C3H infant mice, less than 36 hours old, were inoculated subcutaneously (0.1 cc each) with *Ak leukemic cell suspension* of 20% concentration. Two of these mice died within 19 days of acute transplanted leukemia; the other 2, male No. 304 and female No. 393, remained in apparently good health; they were mated later, and had 2 litters, one born February 14, 1950, consisting of female No. 395 and male No. 295, and another on March 6, 1950, consisting of females Nos. 446, 447, and 448 and male No. 330. The injected father (male No. 304) died when 14 months old without evidence of leukemia, but the injected mother (female No. 393) developed simultaneously "spontaneous" leukemia and also a mammary carcinoma when 16 months old. Among the offspring, male No. 295 of the first litter, developed spontaneous leukemia at 14 months of age, and female No. 447 of the second litter, also died from leukemia at 16½ months of age; at autopsy, 2 small spontaneous mammary carcinomas were also found in this female; her sister No. 446 also developed leukemia at 18 months of age. Of the remaining offspring, female No. 395 died from mammary carcinoma at 13½ months of age, without signs of leukemia. The 2 others are well at 17 and 18 months of age, respectively. (Fig. 1).

Exp. LV 13. C3H(f) female No. 6 and her brother No. 280 were inoculated, when 4 days old, with *Ak embryo cell suspension* (Exp. 1027) on November 29, 1949. The embryo cell suspension was freshly prepared from normal Ak embryos in the manner described before(2); the pregnant Ak female from which the embryos had been removed by surgical procedure was in good health at the time, but her ancestors died from leukemia. Following the inoculation with the embryo-cell suspension, the C3H(f) female No. 6 and her brother No. 280 remained in good health, were mated, and had a litter on March 3, 1950 consisting of 3 females (Nos. 66, 67, and 68). Both parents died without signs of leukemia

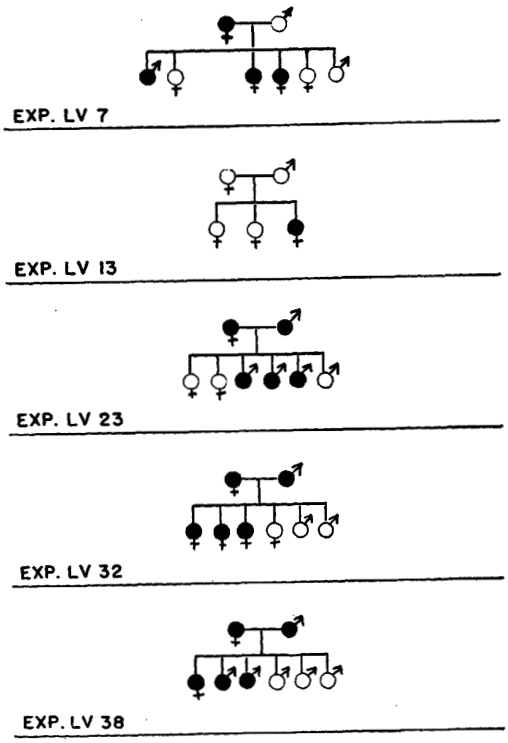

FIG. 1. "Vertical" (from parents to offspring) transmission of the leukemic agent in mice. This figure represents 5 individual experiments designated by their respective symbols. Each experiment consists of 2 generations, parent-mice and their offspring. In all 5 experiments illustrated in this figure, only parents were inoculated (in early infancy) with either centrifugated leukemic extracts (LV 23 and LV 32), with leukemic cell suspensions (LV 7), or with normal Ak-embryo-cell suspensions (LV 13 and LV 38). None of the offspring was treated in any way. Black color indicates the development of "spontaneous" leukemia. It is evident from this figure that 7 of the 10 parents, and 13 of the 27 offspring died from leukemia.

at 16 and 20 months of age respectively. Of the 3 offspring, female No. 68 developed leukemia at 20 months of age; the remaining 2 females are now 21 months old, and apparently in good health.

Exp. LV 23. C3H(f) female No. 16 and her brother No. 289 (of the Exp. 1109-C, Table I), were inoculated, along with 3 other litter mates, when 6 days old, with a *centrifugated leukemic extract*, on January 17, 1950. They remained well, at first, were mated, and had a litter on March 22, 1950 consisting of 2 females (Nos. 97 and 98) and 4 males (Nos. 387, 388, 389, and 390). Both parents died from "spontaneous" leukemia at 18 months of age. Some of their offspring, in turn, later on also developed leukemia as follows: Male No. 389 at 16 months, and males Nos. 390 and 388 at 17 and 18 months respectively. The remaining 1 male and 2 females are in good health at 18 months of age.

Exp. LV 32. C3H(f) female No. 93 and her brother No. 65 were inoculated, when less than 12 hours old, together with 3 other litter mates, (Exp. 1106(2), Table I) with *centrifugated Ak leukemic extract* on January 16, 1950. They remained in good health, and had a litter on April 14, 1950, consisting of males Nos. 122 and 123, and females Nos. 136, 137, 138, and 139. Both parents developed "spontaneous" leukemia at 12 and 18 months respectively (Table I). Some of their offspring developed "spontaneous" leukemia, as follows: Female No. 136, at 16 months of age, and 2 weeks later females Nos. 137 and 138. The remaining 2 males and 1 female are well at 17 months of age.

Exp. LV 38. C3H(f) female No. 118 and her brother No. 96 were inoculated, when less than 12 hours old, with *Ak embryo cell suspension* (Exp. 1155)(2); they remained well and had a litter on May 11, 1950 consisting of 1 female (No. 159) and 5 males (Nos. 134, 135, 136, 137, and 138). Both parents died later with leukemia, female No. 118 at 9 months, and male No. 96 at 8½ months of age. Some of their offspring developed leukemia as follows: Female No. 159 at 14 months of age, and males No. 135 and 136 when 15 months old. Male No. 138 died at 16 months without signs of leukemia, and the remaining 2 males are well at 17 months of age. The results of experiments reported in this series suggest that C3H mice which had been inoculated, in early infancy, with the leukemic agent, apparently present in either Ak leukemic cell suspension (Exp. LV 7), in centrifugated leukemic extracts (Exp. LV 23, and LV 32), or in normal Ak embryo cell suspension (Exp. LV 13, and LV 38), will not only, in some instances, eventually develop leukemia themselves, but may, in addition, transmit the leukemic agent to their offspring. In certain instances, either one, or even both, parents may die without symptoms of leukemia (LV 7 and LV 13), but their offspring may nevertheless develop, and die from, this disease.

Discussion. When our first report was submitted for publication(2), only those mice that had been inoculated with centrifugated leukemic extracts when less than 12 hours old, had developed "spontaneous" leukemia; mice that had been inoculated with the centrifugated leukemic extracts at 2 to 7 days of age were still in good health, most of them being over 12 months of age; thus it appeared that the susceptibility of mice of the C3H(f) line to inoculation with the centrifugated Ak leukemic extracts might be limited to their first few hours of life(2). It was observed subsequently, however, that some of those mice that had been inoculated at 1 to 6 days of age, also eventually developed leukemia, although, among those that had been inoculated after their second day of life, the ultimate development of "spontaneous" leukemia was frequently considerably delayed (Table I). Thus, the activation of the leukemic agent in the carrier-host may depend, among other factors, on the time at which the agent was inoculated; the later the agent was inoculated in the life of the infant host, the later its subsequent activation occurred. This was apparent in experiments in which centrifugated leukemic extracts were inoculated (Table I), as well as in those in which the filtered extracts were injected (Table II).

If the trend indicated by the results of experiments on mice injected at 1 to 6 days of age persists also in mice inoculated at older ages, it may well be that most of those in-

jected with the centrifugated leukemic extracts beyond their first or perhaps second week of life, will die their natural death before having had a chance to develop leukemia; the activation of the dormant leukemic agent in most of these mice, may, theoretically at least, occur beyond their normal life-spans, the normal life of a laboratory mouse usually not extending much over 24 months of age.

The results of experiments on the filtration of the leukemic agent thus far obtained suggest that the Ak mouse leukemia agent is filterable through the Seitz (pad S-1) filter. It is true that only 9 out of the 32 inoculated mice developed leukemia; however 7 mice were 8 days old when they were inoculated, *i.e.*, they were injected rather late in infancy, when the susceptibility to the injection with the leukemic agent is greatly diminished, if at all present(2,3); in the second group, 25 infant mice, less than 12 hours old, were used for inoculation, and 7 of them developed leukemia, but these experiments are of a recent date and at least some of the remaining 18 mice, now in good health at only 7 to 9 months of age, may later develop leukemia.

The question may arise whether the development of leukemia in 9 of the 32 mice that were inoculated with the filtered leukemic extracts was a direct result of the inoculation, or whether it actually was spontaneous, and unrelated to the inoculation. It should be kept in mind, however, that spontaneous leukemia is extremely rare, if it occurs at all, in untreated mice of either the C3H line or of the C3H(f) subline. Over 5,000 males and females of the C3H line and more than 1500 mice of the C3H(f) subline have been observed in this laboratory during the past several years, and less than 0.07% of these animals developed spontaneous leukemia. We have also performed a series of experiments in research projects not related to the present study, in which centrifugated extracts, prepared from normal organs of C3H(f) mice, or other non-leukemic extracts (such as centrifugated extracts prepared from fresh, or inactivated mouse mammary carcinoma, from mouse pulmonary adenoma, human breast carcinoma, or serum collected from treated or untreated rabbits) were injected subcutaneously into 1 to 7 days old suckling C3H infant mice(4). Most of the injected mice were then allowed to live their full lifespan, but none of them has developed leukemia. It appears reasonable to state that spontaneous leukemia is most unusual in mice of either C3H or C3H(f) line, and that it cannot be prompted to develop by means of injecting non-leukemic extracts. It would then logically follow, that those mice which developed leukemia in experiments reported in this paper did so because they were injected with the Ak leukemic agent.

The results of experiments dealing with the development of "spontaneous" leukemia in the offspring of mice that had been inoculated with the leukemic agent suggest that the agent, once inoculated into newborn, suckling mice of a hitherto leukemia-free, but susceptible, line, not only infects most of the inoculated animals, causing in them the development of "spontaneous" leukemia, but also passes from the inoculated carrier-hosts into the offspring, which, in turn, may later also develop, and die from, leukemia. This was evident in 5 experiments in which the parents were inoculated with the leukemic agent, but their offspring were not treated in any way; of the 27 untreated offspring born to these parents, 13, or 48% developed "spontaneous" leukemia at 14 to 20 months of age. Even though, however, some of the inoculated parent-mice, particularly those that had been inoculated when several days old, might have themselves remained free from signs of leukemia for the balance of their lives, they apparently carried, and transmitted, the agent in sufficient quantity to cause the development of "spontaneous" leukemia in some of their offspring; this was evident, for instance, in Exp. LV 13, as well as in other experiments(4). Essentially similar observations have been recently reported by Andervont, who found that when serial dilutions of the mammary tumor agent were administered to young mice, an appreciable number of the inoculated animals failed to develop tumors, but their descendants died from mammary carcinomas(5).

There is no reason to believe that the

3. Gross, L., *Cancer*, 1950, v3, 1073.

4. Gross, L., Unpublished experiments.

leukemic agent would stop at this point its "vertical"*(1,6) trend of transmission; it would be only logical to assume that from this second generation the agent will then pass to the next one, and so forth. Thus, a line of mice hitherto essentially free from this disease, would have changed into a line in which leukemia develops spontaneously in successive generations, as in the case of the leukemic Ak inbred line. A new leukemic inbred line of mice would thus have been originated, at will, by inoculation, in the laboratory. Since, however, the mouse leukemia agent, unlike mouse mammary carcinoma, does not pass from parents to their offspring through mothers' milk(7,8,9,4), but is apparently transmitted directly through the embryos(2), like that of chicken lymphomatosis(10), the investigator would have no means at his disposal of stopping the vertical transmission of mouse leukemia once it has been established in a family of mice.

Summary. 1. When centrifugated extracts of Ak mouse leukemia were inoculated into 16 suckling C3H(f) infant mice less than 12 hours old, "spontaneous" leukemia developed in 8 of them at 8½ to 11 months, and in 5 mice at 14 to 18 months of age. 2. When the centrifugated leukemic extracts were inoculated into 20 C3H(f) infant mice 3 to 6 days old, "spontaneous" leukemia developed in 10 of them, but not before they were 18 months of age. 3. Filtered (Seitz) leukemic extracts were inoculated, in 2 experiments, into 7 C3H(f) infant mice 8 days old, and 2 of them developed leukemia at 23 and 27 months respectively. In 5 additional experiments, leukemic filtrates were inoculated into 25 C3H(f) infant mice less than 12 hours old, and 7 of them developed "spontaneous" leukemia at 6½ to 9 months of age. These results suggest that the Ak mouse leukemia agent is filterable. 4. In 5 experiments, suckling infant C3H or C3H(f) mice were inoculated with either centrifugated leukemic extracts, leukemic cell suspensions, or with normal Ak-embryo-cell suspensions. After the inoculated infant mice have grown up and reached sexual maturity, they were mated, Of the 10 inoculated parents, 7 eventually developed "spontaneous" leukemia at 9 to 19 months of age. Of the 27 untreated offspring, 13, or 48%, have thus far developed "spontaneous" leukemia at 14 to 20 months of age. The results of these experiments suggest that the mouse leukemia agent is transmitted from parents to their offspring. The transmission of the agent occurs most probably directly through the embryos(2,4). 5. The incidence of spontaneous leukemia among the untreated males and females of the C3H or C3H(f) colonies of mice in our laboratory has been, during the past several years, less than 0.07%.

5. Andervont, H. B., *J. Nat. Cancer Inst.*, 1950, v11, 545.

6. Gross, L., *Cancer*, 1951, v4, 626.

7. MacDowell, E. C., and Richter, M. N., *Arch. Path.*, 1935, v20, 709.

8. Barnes, W. A., and Cole, R. K., *Cancer Research*, 1941, v1, 99.

9. Furth, J., Cole, R. K., and Boon, M. C., *Cancer Research*, 1942, v2, 280.

10. Cottral, G. E., *Proc. 53rd Ann. Meet. U. S. Livestock San. Assn.*, 1950, 183.

Received August 29, 1951. P.S.E.B.M., 1951, v78.

Mechanisms of Amethopterin Resistance in Leukemia. I. Effects of Weak Folic Acid Antagonists on Mouse Leukemias. (19069)

JOSEPH H. BURCHENAL,* SHIRLEY F. JOHNSTON, AND GEORGIA B. WARING.

From the Division of Experimental Chemotherapy of the Sloan-Kettering Institute for Cancer Research, Memorial Cancer Center, New York City.

In the 20% to 50% of children with acute leukemia whose disease initially responds to amethopterin (4-amino-N^{10}-methyl PGA), the eventual failure of therapy is usually due to the development of resistance to this agent by the leukemic cell(1,2). For this reason experimental studies of the mechanism of this resistance have been undertaken in the hope