TABLE I. Effect of Pentobarbital Sodium Anesthesia upon Volume Blood Flow, Mean Arterial Pressure and Pulse Rate.

	Pre-anesthetic control	% change from control, min. after inj. .5	1	2	5	15
Flow in cc/min.	45	+119	+110	+62	+13	0
S.D.		97	88	29	25	—
S.L.*		1%	1%	1%	18%	—
Pressure, mm/Hg	130	—16	—35	—33	—17	—12
S.D.		11	6	17	14	10
S.L.*		1%	1%	1%	2%	3%
Pulse rate/min.	110	+34	+36	+41	+39	+39
S.D.		25	20	26	25	25
S.L.*		1%	1%	1%	1%	1%

* Level of significance.

the necessity of being cognizant of the effect of pentobarbital sodium anesthesia *per se* upon volume blood flow and arterial pressure in any experiments in which measurement of changes in these factors are pertinent to a study. Attention should also be called to the finding that supplemental administration of the drug for the purpose of maintaining satisfactory surgical anesthesia was also followed by transient changes of a significant magnitude in blood flow and arterial pressure. A sufficient period of time should be allowed to elapse following anesthetic administration either for recovery of blood pressure and flow to pre-anesthetic control values or to a new steady state. Our findings indicate that considerable variation exists in the time required for such readjustments to be made in experiments on different animals.

Summary. A study was made with the aid of continuous recording of the effects of an anesthetic dose of pentobarbital sodium and of supplemental doses of this drug required for the maintenance of surgical anesthesia upon arterial pressure, pulse rate and volume blood flow through the femoral artery of dogs. Following the administration of this anesthetic there was an immediate increase in volume blood flow and a fall in mean arterial pressure. Recovery to near pre-anesthetic control values usually occurred within 5 to 15 minutes. However, in some experiments recovery was not apparent for as long as 30 minutes after drug administration. Pulse rate remained increased throughout the duration of anesthesia.

1. Richter, H. G., and Oughterson, A. W., *J. Pharm. Exp. Therap.*, 1932, v46, 335.

2. Tabern, D. L., *Clin. Med. Surg.*, 1930, v37, 809.

3. Morrison, J. L., Walker, H. A., and Richardson, A. P., *Arch. internat. Pharmacodyn. Therap.*, 1950, v82, 53.

4. Richardson, A. W., Randall, J. E., and Hines, H. M., *J. Lab. Clin. Med.*, 1949, v34, 1706.

Received November 18, 1952. P.S.E.B.M., 1953, v82.

Adrenals and Anterior Hypophysis in Leukemogenesis of Mice.* (20006)

MARTIN SILBERBERG, RUTH SILBERBERG, AND MARION OPDYKE.

From the Snodgras Laboratory, Hospital Division, City of St. Louis, Mo.

In male castrate mice of strain A, anterior hypophyseal transplants significantly increased the incidence of malignant lymphoma (1). This result was tentatively explained as due to hyperadrenalism caused by the grafted hypophyses: Hyperadrenalism leads to depletion of lymphoid tissue, and protracted atrophy of the latter may be followed by hyperplasia and eventually neoplasia(2-4). On the

* Aided by a Grant from the Brant Fund of the Am. Med. Assn.

TABLE I. Incidence of Leukemia and Leukemia Age.

Series	No. of mice			% of leukemia	Leukemia age, mo	
	Total	Of leukemia age	With leukemia		Mean	Range
Castrates	39	32	2	6.3	17.5	13-22
Castrates bearing adrenal grafts	38	30	4	13.3	12.8	10-18
Castrates bearing adrenal and hypophyseal grafts	40	32	8	25	12.5	8-19
Castrates bearing (1) hypophyseal grafts	28	28	7	25	16.6	9-20

other hand, in adrenalectomized rats adrenal transplants inhibited growth of transplanted lymphatic leukemia(5); while, likewise in rats, lymphosarcoma was observed following treatment with anterior hypophyseal growth hormone(6). In order to further test the role of adrenals and hypophyses in leukemogenesis, the following experiments were carried out.

Material and methods. One hundred and twenty newborn mice of the closely inbred strain A, raised in our laboratory, were orchidectomized and after weaning divided into 3 groups of 40 animals each. *Series I:* The castrates received no further treatment. *Series II:* The castrates received subcutaneous transplants of 4 adrenals; 8 mice received adrenals from male, 27 from female, and 5 from both male and female donors. *Series III:* The castrates received 4 adrenals and 4 hypophyses; 10 mice received adrenals from male, 28 from female, and 2 from both male and female donors; 7 mice received hypophyses from male, 25 from female, and 8 from both male and female donors. All grafts were obtained from 1- to 3-month-old donors closely related to the recipient. The animals were fed a stock diet of Purina Laboratory Chow and water *ad libitum.* Two mice of each series were sacrificed at the ages of 2, 4, 5, or 7 months; later the animals were killed as their condition warranted it. Some mice died unexpectedly, and in a few of these autolysis was too far advanced to permit microscopic examination of the tissues. These were excluded from the investigation. Details may be seen from the table. At autopsy, pieces of internal organs, the legs, mammary glands, thyroids, hypophyses, adrenals and transplants, when present, were removed, fixed in Bouin's solution and embedded in paraffin. Semiserial sections stained with hematoxylin and eosin were prepared for microscopic studies. Some hypophyses and hypophyseal grafts were fixed in Regaud's solution for cytological and histochemical studies.

Results. Only those cases were classified as leukemia that could be diagnosed grossly and that microscopically showed extensive infiltrations of lungs, liver, spleen, heart and kidneys.

Series I: Among 39 castrates 32 lived longer than 8 months. This was the earliest age at which leukemia was observed in any of our animals. Of these mice only two (6.3%) had lymphatic leukemia at the mean age of 17.5 months, as compared with a 3.6% incidence at a mean age of 16.9 months in our controls. *Series II:* Of 38 castrates bearing adrenal grafts 30 reached leukemia age, and of these 13.3% had lymphatic leukemia at a mean age of 12.8 months. In 20 of these mice (66.7%) living adrenal grafts were found microscopically. *Series III:* Of 40 castrates carrying adrenal and hypophyseal grafts 32 reached leukemia age, and of these 25% had developed lymphatic leukemia at a mean age of 12.5 months. In 19 of these mice (59.4%) adrenal and in 17 mice (53.1%) hypophyseal grafts were identified microscopically.†

Fate of grafts in leukemic mice. Grafts were found in 3 of 4 mice bearing adrenal transplants (Fig. 1, 2). Some grafts underwent hyperplastic changes similar to those

† The actual number of mice showing hypophyseal grafts was probably higher, since tissue grossly most suggestive of "takes" was retained for cytological studies which will be reported at a later date.

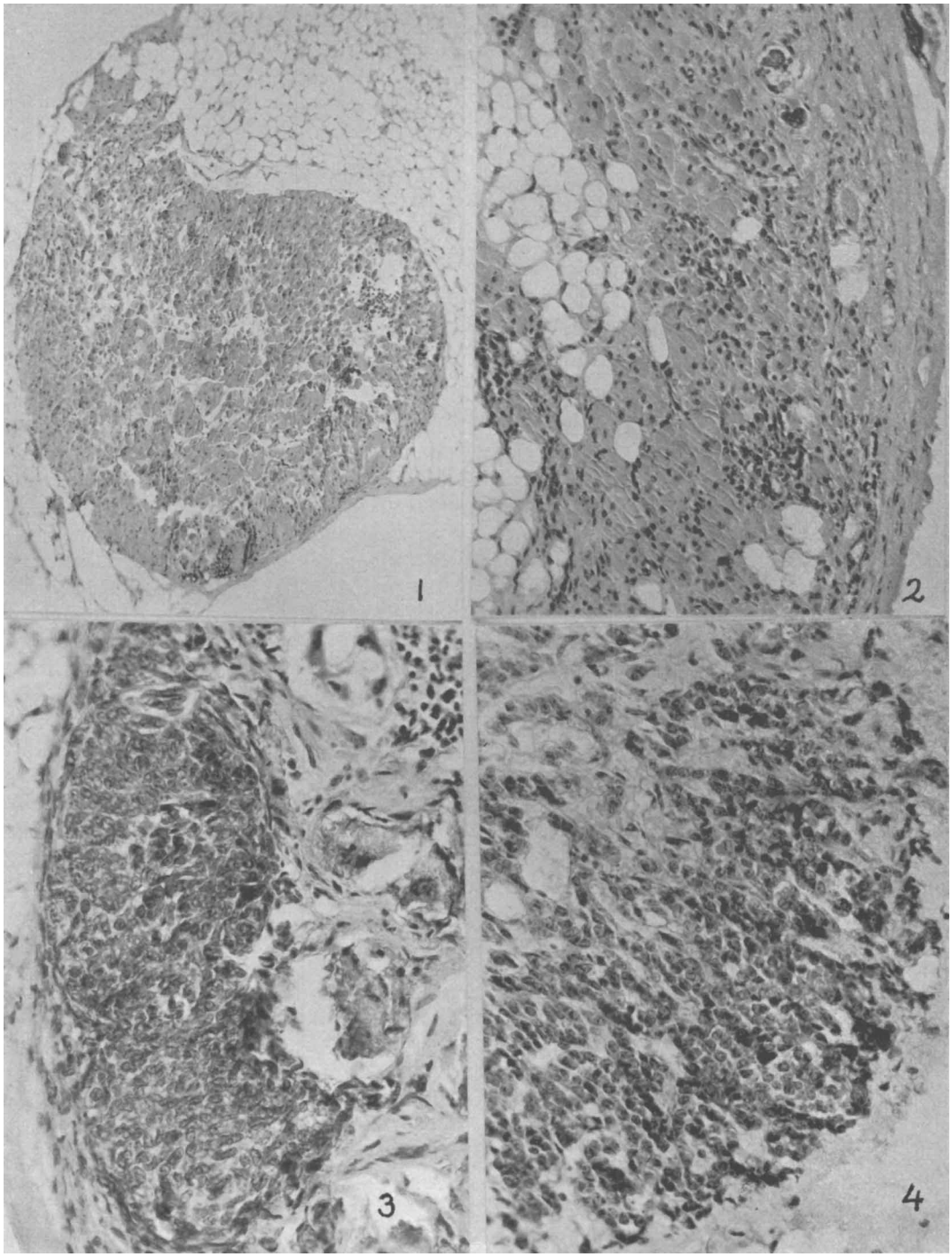

FIG. 1. Section through an adrenal graft 10 mo after transplantation. Magnification × 75.

FIG. 2. Section through an adrenal graft 10 mo after transplantation. The mouse has also received hypophyseal grafts. Magnification × 290.

FIG. 3. Section through another area of the transplant shown in Fig. 2 demonstrating a hyperplastic focus in the adrenal cortex. Magnification × 300.

FIG. 4. Section through a hypophyseal graft 18 mo after transplantation. Magnification × 300.

seen in the animals' own adrenals (Fig. 3). Of 8 leukemic mice carrying adrenal and hypophyseal grafts, adrenal tissue was recovered in 6 and hypophyseal grafts in 5 animals (Fig. 4). Obviously, there was no difference in the stimulating effects of male or female grafted glands.

Structure of adrenals in leukemic animals. Of the 2 untreated leukemic castrates one showed slight focal cortical hyperplasia and the other one cortical adenomas. Of the 4 leukemic castrates bearing adrenal grafts, one showed resting adrenal cortex, two slight focal cortical hyperplasia and one cortical adenomas. Of the 8 leukemic castrates bearing adrenal and hypophyseal grafts, one showed resting adrenal cortex, one slight focal, and one diffuse cortical hyperplasia, while the remaining 5 animals showed cortical adenomas.

Structure of mammary glands in leukemic animals. In order to determine whether or not the adrenal changes were associated with the production of estrogen, the mammary glands were investigated. In none of the leukemic mice was acinar growth stimulation present. Of the total of 14 leukemic mice, 10 had resting mammary glands, and only 4 showed slight ductal growth stimulation. Therefore, any estrogenic effect if present at all would have been slight at best and could hardly account for leukemogenesis.

Discussion. Orchidectomy performed in newborn mice of strain A increased the incidence of leukemia from 3.5% in untreated controls to 6.3%. In castrates bearing adrenal transplants the incidence of leukemia was further increased to 13.3%, and the mean age of the animals at the time of leukemia development was advanced by 4.7 months as compared with that seen in untreated castrates. This finding which represents a considerable shortening of the latent period may be attributed to the action of the grafted adrenals. As reported previously, in mice of strain A orchidectomized at the age of one month and bearing only hypophyseal transplants, a 25% incidence of leukemia was observed at a mean age of 16.6 months(1). In newborn castrates carrying adrenal and hypophyseal transplants, the incidence of leukemia was likewise 25%. However, the induction period was 4.1 months shorter than in castrates carrying hypophyseal grafts only. This finding again points to the role of the adrenal grafts in shortening the latent period of leukemia. None of the leukemic animals showed acinar growth of the mammary gland, and none of the non-leukemic animals developed mammary cancer, although some of the latter mice showed acinar hyperplasia. Therefore, the adrenal cortex did not produce large amounts of estrogen, and thus estrogen might not have played the primary role in this type of leukemogenesis. On the other hand, the present results do not rule out a direct effect of the hypophysis on hemopoietic tissues; however, the adrenal cortex seems to take part in the evolution of the leukemic process.

Summary. In newborn mice of strain A, orchidectomy slight increased the incidence of leukemia. In such castrates, adrenal transplants doubled the incidence and conspicuously advanced the onset of the disease. Hypophyseal transplants made in conjunction with adrenal grafts increased the incidence of leukemia 4 times over that seen in non-grafted castrates. Prolonged hyperpituitarism associated with hyperadrenalism apparently exerted this leukemogenic effect.

1. Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 547.

2. Dougherty, T. F., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1943, v53, 132.

3. Gardner, W. U., Dougherty, T. F., and Williams, W. L., *Cancer Res.*, 1944, v4, 73.

4. a. Furth, J., and Boon, M. C., 1945, *A.A.A.S. Cancer Research Conference,* 129. b. *Proceed. Inst. Med.,* Chicago, 1952, v19, 5

5. Murphy, J. B., and Sturm, E., *Cancer Res.*, 1950, v10, 191.

6. Moon, H. D., Simpson, M. E., Li, Ch. H., and Evans, H. M., *Cancer Res.*, 1950, v10, 297.

Received November 18, 1952. P.S.E.B.M., 1953, v82.