11129

Attempt to Modify Growth, Development and Tumor Incidence in Mice with Thymus Gland Extracts.

GEORGE VAN S. SMITH AND E. ELIZABETH JONES.

From the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass. (The Reginald W. Bird Endowment.)

Spontaneous mammary cancer in mice is predominantly a disease of mature or old females and is influenced in many strains by their breeding activity. It, therefore, becomes of great interest to modify reproductive activity, development and growth in strains of mice of known rates of cancer susceptibility to determine the relationship between the chronological and physiological age of tissues and the appearance of cancer.

The biologic effects of thymus gland extracts on rats reported by Rowntree, Clark and Hanson[1] and Rowntree[2] were precisely of the nature we desired to produce in mice. These were: increase in number and size of litters and birth weight of offspring; and, in the third generation of treated rats, precocious development accompanied by early eruption of teeth, appearance of fur, opening of eyes, descent of the testes or opening of the vagina and earlier sexual maturity.

The present experiments were undertaken with the Line A strain of albino mice, in which the tumor incidence shows a high correlation with breeding activity. Approximately 5% of virgin females develop tumors at 11 months of age. In bred females, which develop tumors at an average age of 15 months, the percentage of tumors increases with the number of litters borne: 12.56% in those with 1; 24.44% in those with 2; and 34.21% in those with 3 litters.

Three possible effects of thymus gland extract injections presented themselves. First, with an increase in the number of litters, a rise in tumor incidence might be anticipated; on the other hand, if the extract produced maturation and differentiation of cells, such an effect might either prevent the appearance of tumors, which are typically of undifferentiated cells, or, by bringing about precocious physiological maturity, hasten the onset and increase the number of tumors.

Extracts of thymus glands from young calves were prepared in this laboratory by the method then employed by Rowntree (personal communications, Dec., 1935) and their potency in terms of reduced

1 Rowntree, L. G., Clark, J. H., and Hanson, A. M., *J. A. M. A.*, 1934, **103**, 1425.
2 Rowntree, L. G., *J. A. M. A.*, 1935, **105**, 592.

TABLE I.
Females Receiving Thymus Gland Extract in F_1, F_2, F_3 Generations from Treated Parents.

Generation	No. ♀s bred	Died before 3 mo. without offspring	Survived after 3 mo.	Fertile		No. of litters	No. of offspring		Sterile	
				No.	Range in age at death, mo.		♂	♀	No.	Range in age at death, mo.
F_1	7	2	5	4	8-15	7	23	15	1	
F_2	14	6	8	2	3- 6	2	6	3	6	1-24
F_3	3	0	3	0		0			3	14-22
Totals	24	8	16	6		9	29	18	10	

glutathione content determined. Extracts containing from 28 mg % to 110 mg % reduced glutathione were administered by intraperitoneal injections of 0.5 cc 3 times a week from weaning throughout the reproductive period. They were stored in small sterile vials in the refrigerator. An opened vial was never used for more than 3 weeks and no extracts were more than 12 weeks old when injected. The material was found to be toxic if given at more frequent intervals.

After a number of preliminary experiments, a litter of 3 females and 1 male was selected to start a line, all females and males (*i. e.*, those used for breeding) of which received thymus gland extract in each generation. Of the 3 original females, 1 died at 4 months, with no offspring, 1 at 6 months, having had 1 litter of 4, and the third at 21 months, having had 3 litters totalling 15 young. This mouse developed a tumor at 20 months of age. The progeny in succeeding generations are listed in Table I. It will be seen that contrary to the expected increase in number and size of litters, the fertility in the second generation decreased markedly, and only 3 third-generation females were born. These 3 females were sterile.

Birth weights and growth increments of young in the F_1, F_2 and F_3 generations were well within the normal range as compared with the controls. There was no evidence of developmental precocity.

Mammary gland tumors appeared at 20 months in the F_0 female which had had 3 litters and at 24 months in 1 sterile F_2 female. Leukemia developed at 14 months in 1 sterile F_3 female. Thymus gland extract, therefore, did not inhibit the onset of spontaneous tumors in mice which had received it over a considerable part of their life span. Because of decreased fertility, the number of animals available for the observation of tumor incidence was too small to permit drawing any other conclusions. Nineteen females of the experimental group survived to tumor age and only 3 of these had been fertile. Injections of extract into 1 female with spontaneous mammary cancer had no noticeable effect on the tumor.

From the findings of Rowntree, *et al.*, the potency of thymus gland extract appeared (at the time these experiments were started) to be linked with its sulfhydryl content. Based upon this consideration, a parallel experiment was carried out with *p*-thiocresol.* This choice was determined by the reports of Reimann[3] and Reimann and Ham-

* The *p*-thiocresol was obtained from the Eastman Kodak Company and made up as described by Reimann;[2] *i. e.*, 0.01 g dissolved in 5 cc of 95% ethyl alcohol, to which was added 100 cc distilled water, a dilution of approximately 1:10,000. Intraperitoneal injections of 0.5 cc of this solution were given 3 times weekly.

3 Reimann, Stanley P., *J. A. M. A.*, 1930, **94**, 1369.

mett[4] concerning its stimulating effect on wound healing and cell proliferation, and by the work of Hammett[5, 6] on the action of the SH-radical in stimulating mitosis.

In this experiment ρ-thiocresol was injected into 5 generations of Line A albino mice from weaning at 21 days of age throughout their breeding period. The material was not toxic and the mice withstood the injections well. No change in fertility, growth or development ensued and there was no significant difference in cancer incidence in the 5 generations of treated animals or between treated mice and stock Line A females of similar breeding history.

Injections of ρ-thiocresol were likewise given to 10 mice with inoculated tumors (tumor 478, an adenocarcinoma which grows progressively on inoculation into Line A strain mice) to test the effect of the material on the growth of tumor cells. Treatment was begun while the tumors were still small. Daily injections of 0.5 cc ρ-thiocresol in 1:10,000 dilution were given intraperitoneally to 5 and subcutaneously to 5. After 1 week, during which the tumors had grown at their normal rate, injections of ρ-thiocresol in dilution of 1 :5000 were given in the same manner but on alternate days for a period of 10 days. All tumors grew steadily and there was no evidence of either stimulation or inhibition.

Summary. Thymus gland extract was without stimulating effect on growth or development in Line A albino mice when injected in doses of 0.5 cc 3 times weekly from the time of weaning throughout the reproductive period. A marked lowering of fertility occurred in the second generation from treated parents and grandparents. There were only 3 third-generation females and these were sterile. This unexpected decrease in fertility resulted in too small a number of females surviving to tumor age to permit any conclusions as to the effect of thymus extract upon tumor incidence other than that it did not prevent their appearance or affect their growth.

ρ-Thiocresol was without effect on fertility, growth or development of Line A albino mice and did not modify the occurrence of spontaneous tumors. It did not influence the growth of inoculated tumors.

[4] Reimann, Stanley P., and Hammett, Frederick S., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 20.

[5] Hammett, Frederick S., *Protoplasma*, 1931, **13**, 331.

[6] Hammett, Frederick S., *Arch. Path.*, 1929, **8**, 575.