mix.

This observation was repeated in series 4 and in addition each of the 4 amino acids was added separately to the basal diet. Of the single amino acid supplements tested only tryptophan produced an appreciable betaine transmethylase activity.

The growth data given in Table I are of considerable interest. The basal diet permitted approximately half-normal growth. Supplementation of the basal diet with 10% casein, complete amino acid mix, yeast, or liver extract improved the growth rate. Supplementation of the basal diet with threonine resulted in growth equal to that obtained with rats fed chow. With respect to growth, methionine is the first limiting amino acid in alpha protein(5) and the present experiments indicate that threonine is the second limiting amino acid.

It is apparent from the data in Table I that there is no correlation between the effects of the various supplements on growth and on betaine transmethylase activity. Thus tryptophan supplementation resulted in considerable enzyme activity but actually depressed growth. Yeast extract improved growth considerably but was ineffective in stimulating the synthesis of the betaine transmethylase enzyme.

These observations suggest that the amino acid pattern required for the synthesis of this particular liver enzyme must be considerably different from the pattern required for growth of the animal. Also, the observation that some of the diets permitted good growth but allowed negligible synthesis of betaine transmethylase suggests that when rats are fed methionine the enzyme is not vital to the animal's economy. Although the observation periods in the present experiments were of short duration, in other experiments rats have been fed the basal diet for 5 months and continued to grow at a rate of approximately 2 g per day throughout this period.

Summary. 1. Rat liver betaine transmethylase varies strikingly with the amino acid composition of the diet. 2. The pattern of amino acids required for synthesis of this enzyme is considerably different from the pattern required for growth of the animal. 3. When rats are given methionine, betaine transmethylase does not appear to be necessary for growth.

1. Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, 1947, v169, 247.

2. Dinning, J. S., Keith, C. K., and Day, P. L., *ibid.*, 1951, v189, 515.

3. Oginsky, E. L., *Arch. Biochem.*, 1950, v26, 327.

4. Hubbell, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutr.*, 1937, v14, 273.

5. Dinning, J. S., Payne, L. D., and Day, P. L., *ibid.*, 1951, v43, 525.

Received October 26, 1953. P.S.E.B.M., 1953, v84.

Effect of Antigonadotrophic Serum on Testes of A-Strain Mice Treated with Estrogen.* (20692)

CHARLES A. ELY. (Introduced by W. U. Gardner.)

From Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

The development of interstitial cell tumors in A-strain mice after prolonged estrogen treatment has been demonstrated repeatedly(1-4). Gardner(5) has suggested that these tumors arise as the result of enhanced secretion of interstitial cell stimulating hormone (ICSH) by the pituitary gland under the influence of low doses of estrogen rather than from a direct effect of estrogen upon the testis. The present report presents the results of an attempt to test this hypothesis by nullifying the presumed excessive ICSH secretion with antigonadotrophic serum.

Materials and method. For the production

* Aided by grants from the Anna Fuller Fund, the Damon Runyan Memorial Fund for Cancer Research, Inc., and the Society of Sigma Xi.

of the antiserum, a crude sheep pituitary extract† prepared according to the method of McShan and Meyer(6) was used. Adult rabbits were injected with amounts of the extract equivalent to 250 or 500 mg of acetone dried powder. This quantity is sufficient to increase the ovarian weights of immature female rats from the normal of 10-15 mg to 60-80 mg when assayed according to the method described by McShan and Meyer(6) and used by Deutsch *et al.*(8). Injections were given subcutaneously daily 5 times per week for 8 weeks, after which a series of 5 injections was carried out every third week only. At the end of the eighth week and at irregular intervals thereafter, the rabbits were bled from the central artery of the ear or by heart puncture. After cooling, the serum was recovered by centrifugation and stored in the frozen state until used. All serum assays for antigonadotrophic activity followed a method essentially similar to that described by Deutsch *et al.*(8). Only sera with demonstrable antigonadotrophic activity were used.‡ *Male A-strain* mice 30-40 days of age were employed. These were pen-bred animals, the original stock of which was obtained from Dr. W. U. Gardner of Yale University. Injections of 16.5 μg of estradiol benzoate§ in .05 ml of sesame oil were given subcutaneously weekly for 22 weeks. It was anticipated that within this interval the interstitial cell changes characteristic of estrogen treated animals would have become apparent.

At the end of this period, animals were divided into 4 experimental categories which continued to receive estrogen as described above. In addition to the estrogen, 5 daily subcutaneous injections per week of antigonadotrophic serum, normal rabbit serum, or pituitary extract were administered. *Normal rabbit* serum was chosen as the material which would most nearly fulfill the requirements of a control substance for the antigonadotrophic serum; inclusion of the series receiving pituitary extract was intended to provide a means of comparing the effect of an actively developed resistance with that of the passive resistance conferred by the rabbit antigonadotrophic serum.

The 4 groups of animals were treated as follows: Group 1 received .2 ml of antigonadotrophic serum, Group 2, .2 ml of normal rabbit serum, Group 3, an amount of pituitary extract equivalent to 25 mg of acetone dried pituitary powder in .25 ml of saline, and Group 4 received no treatment additional to the estrogen. A *fifth group* was made up of animals sacrificed at the outset of the experiment after 15 to 22 injections of estradiol, thus providing a means of evaluating the degree of interstitial cell hypertrophy and hyperplasia at the time injection of additional materials was initiated. *Injections* were continued until the animals became moribund, at which time autopsies were performed. The period of treatment extended from 3 injections of estradiol beyond the initial 22 injections in conjunction with 15 injections of either normal serum, antiserum, or pituitary extract to 10 injections of estradiol with approximately 50 injections of either of the added materials. Most animals, however, survived from 5-7 injections of estradiol in addition to 25-35 injections of the sera or extract (Table I). At *autopsy*, testes, seminal vesicles, and spleen were weighed and fixed in Bouin's solution, and in representative cases the adrenal, thyroid, and pituitary glands were similarly preserved. The testes were serially sectioned and lengths chosen at regular intervals along the ribbon for mounting. Either the hematoxylin and eosin or the periodic acid-Schiff method was used for staining. Approximate estimates of the degree of hypertrophy and hyperplasia were made by tracing selected sections at magnification of 60 X. The modified areas were measured with a planimeter and the total volume of the hypertrophied and hyperplastic cells was calculated for each

† The author is indebted to Dr. W. H. McShan of the Department of Zoology, University of Wisconsin, for donating a portion of the pituitary extract and for generous assistance with the preparation of the remainder. The extract contains negligible amounts of lactogenic activity, but measurable thyrotrophic activity. On the basis of assays of short duration there appears to be little adrenotrophic or growth hormone activity(7).

‡ Sera prepared from extracts similar to those employed in this study inhibited endogenous gonadotrophins in rats under varied experimental conditions (9-11).

§ The estradiol benzoate was supplied through the courtesy of Dr. C. H. Sullivan of Ciba Pharmaceutical Products, Inc.

TABLE I. Effect of Antigonadotrophic Serum on the Development of Interstitial Cell Hypertrophy and Hyperplasia.

Group	Treatment* (No. inj.)	No. animals†	Body wt (g)	Spleen wt (mg)	Testis wt (mg)	No. testes sectioned	Interstitial cell volumes			
							% >.1 mm³	Mean (mm³)	% <.1 mm³	Mean (mm³)
1	Estrogen 3-10, antiserum 15-48	45	24.6 ± .71‡	321.6 ± 16.3	31.3 ± 1.78	53	24	1.65 ± .75	76	
2	Estrogen 3-13, Normal serum 15-59	33	24.5 ± .85	366.5 ± 23.3	77.4 ± 3.38‖	36	86‖	2.10 ± .56¶	14	<.1§
3	Estrogen 3-10, pituitary extr. 13-46	34	23.9 ± .62	248.6 ± 16.8	64.7 ± 4.18‖	42	59‖	1.00 ± .32¶	41	
4	Estrogen 3-10	27	26.6 ± .52	234.3 ± 15.5	99.1 ± 5.21‖	46	50‖	1.73 ± .70¶	50	
5	**	23	22.5 ± .64	160.3 ± 14.9	97.4 ± 3.14‖	25	32¶	.51 ± .21¶	68	

* Treatment begun after 22 inj. of estradiol. † Some animals included which had been dead several hr before autopsy. ‡ SE = $\frac{SD}{\sqrt{N}}$.

§ Majority of cases showed either no evidence of stimulation or too small to measure. ‖ $t = >2.5$, $p = <.02$. ¶ $t = <2.5$, $p = >.05$.

** Autopsied after 15 to 23 inj. of estradiol.

testis. Since the testes of an individual do not necessarily respond to the same extent, each testis was treated separately in measuring the degree of interstitial response. The total number of testes measured and the per cent of the total number showing volumes greater and less than .1 mm³ are given in Table I.

Results. The testes of the animals receiving estrogen and antiserum were greatly reduced in weight. Typical examples had small closely packed tubules. The internal organization of the tubules was destroyed. There were no mature sperm. Often the tubular content was limited to Sertoli cells, but a few scattered spermatogonia and spermatocytes were present as a rule. The extensive areas of hypertrophied and hyperplastic tissue typical of estrogen treated animals were totally lacking or represented by clusters of atrophied interstitial cells (Fig. 2, 5). Only 24% of the testes in this group had interstitial cell volumes greater than .1 mm³, whereas in the remaining 3 groups of treated animals 50 to 86% of the cases fell within this volume range.

The injection of normal serum or pituitary extract caused a reduction in testicular weight below that of animals which were given estrogen alone, but the effect was not as marked as in the case of antiserum treatment. Tubular atrophy in these groups was not as advanced as in the antiserum treated group, but more so than in the group receiving estrogen only. Tubules with mature sperm and intermediate stages of the spermatogenic series were frequently encountered. As indicated above, areas of interstitial hyperplasia and hypertrophy were numerous and extensive.

Sections from a normal serum treated specimen are shown in Fig. 1 and 4. A large area of hypertophied and hyperplastic interstitial cells is present. Tubular damage, as is usual in the vicinity of areas of extensive hypertrophy and hyperplasia, is well advanced. An example taken from Group 4, which received continued injections of estrogen only, is shown in Fig. 3 and 6. Many essentially normal tubules are seen in this testis. A small area of modified interstitial cells may be seen at the right.

The cytological changes characteristic of certain strains of mice when treated with

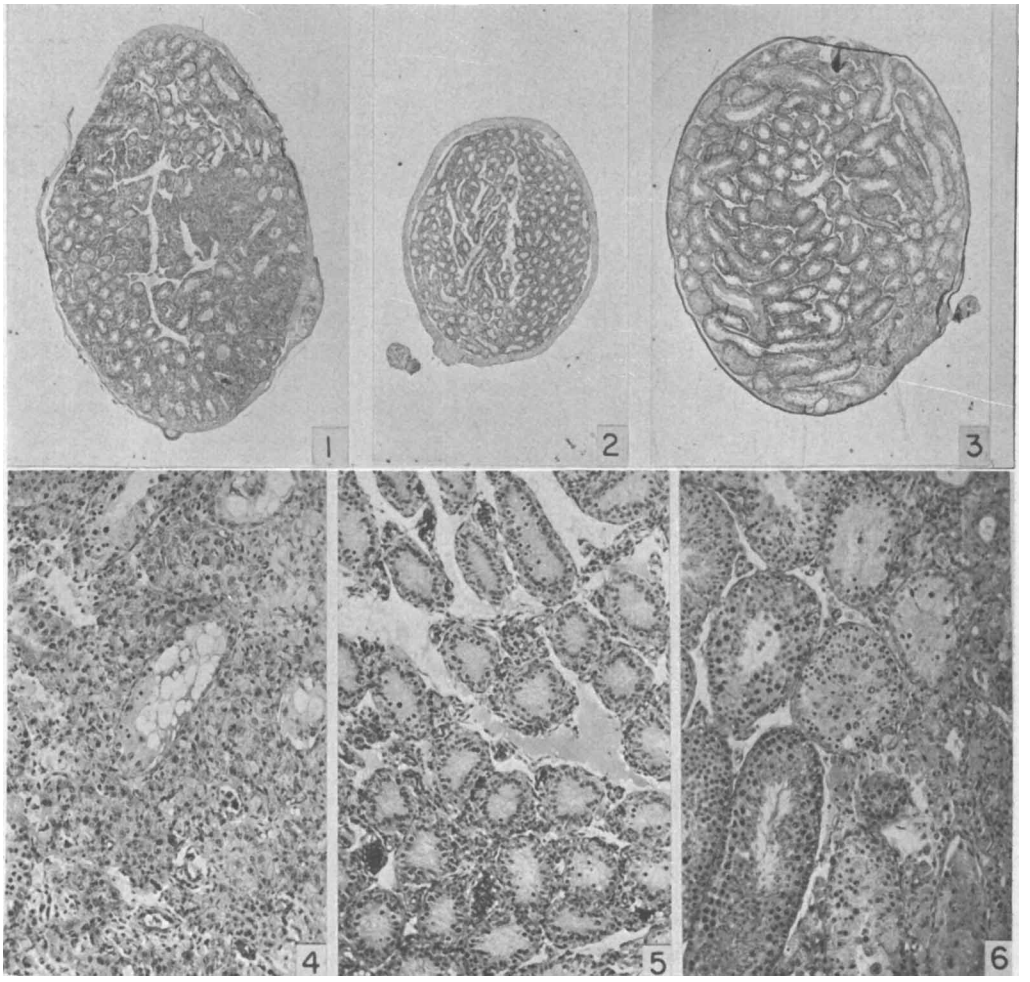

FIG. 1. Testis after 5 weekly injections of estradiol benzoate and 25 daily injections of normal rabbit serum in addition to 22 preliminary injections of estradiol benzoate. × 14. Total vol. of pretumorous area was 4.32 mm³.

FIG. 2. Testis after 8 weekly injections of estradiol benzoate and 37 injections of antiserum in addition to 22 preliminary injections of estradiol benzoate. × 14. There were no pretumorous areas of interstitial cells in this testis.

FIG. 3. Testis after 5 weekly injections of estradiol benzoate in addition to the 22 preliminary injections of the same material. × 14. Total vol. of pretumorous area 0.22 mm³.

FIG. 4. Same as Fig. 1. × 67.

FIG. 5. " " Fig. 2. " .

FIG. 6. " " Fig. 3. " .

estrogen have been described frequently(1-4). No striking departure from these observations was noted when modified interstitial cell areas in the testes from all experimental groups were studied.

The small series of adrenal, thyroid, and pituitary glands examined without recourse to quantitative procedures failed to show any marked differences between the group treated with normal serum and that treated with antiserum.

The body and spleen weights of groups receiving comparable treatment were not found to be significantly different.

Discussion. Until the comparatively recent work of Biskind and Biskind(12), Moon *et al.* (13,14) and of Griffin, Rinfert, and Corsigilia (15), there has been little experimental evidence to show that the secretions of the pituitary gland play an important role in tumor

induction and growth. However, data accumulating from work in these laboratories has demonstrated that the role of the pituitary gland in tumorigenesis may be an important one. It is believed that the data presented here may be interpreted as further evidence of the participation of the pituitary gland in the development of neoplasms, inasmuch as it appears to support the hypothesis that the pituitary gonadotrophin, ICSH, is responsible for the induction of interstitial cell tumors.

The alternative possibility that the apparent suppression of early interstitial cell changes by antigonadotrophic serum is an aspecific effect is not to be overlooked. The lack of significant differences in spleen and body weights between the two groups of animals treated with normal rabbit serum and antiserum has been taken to indicate similarity of response to the rigors of the experimental procedure. However, further investigation into this aspect of the problem is needed and is to be carried out in connection with a study of the efficacy of antigonadotrophic serum when applied to fully developed interstitial cell tumors.

Summary. 1. Male A-strain mice treated simultaneously with estrogen and normal rabbit serum developed extensive interstitial cell hypertrophy and hyperplasia. Animals receiving an antigonadotrophic serum prepared by injecting a crude sheep pituitary extract into rabbits failed to show the same intensive interstitial cell changes. 2. These results have been interpreted as indicating that the effect of the excessive ICSH, presumed to be elicited from the pituitary gland under the influence of estrogen, has been nullified by the antigonadotrophic serum. The consequent suppression of the interstitial cell hypertrophy and hyperplasia, which normally occurs in this strain of mice when treated with estrogen, provides further evidence that the pituitary gland participates in growth and development of interstitial cell tumors. The possibility that the antiserum exerts an aspecific toxic effect is considered.

1. Hooker, C. W., Gardner, W. U., and Pfeiffer, C. A., *J.A.M.A.,* 1940, v115, 443.

2. Bonser, G. M., and Robson, M. J., *J. Path. and Bact.,* 1940, v51, 9.

3. Shimkin, M. B., Grady, H. G., and Andervont, H. B., *J. Nat. Cancer Inst.,* 1941, v2, 65.

4. Hooker, C. W., and Pfeiffer, C. A., *Cancer Research,* 1942, v2, 759.

5. Gardner, W. U., *ibid.,* 1948, v8, 397.

6. McShan, W. H., and Meyer, R. K., *J. Biol. Chem.,* 1943, v151, 259.

7. McShan, W. H., Personal communication.

8. Deutsch, H. F., McShan, W. H., Ely, C. A., and Meyer, R. K., *Am. J. Physiol.,* 1950, v162, 393.

9. Kupperman, H. S., Meyer, R. K., and McShan, W. H., *Endocrin.,* 1941, v29, 525.

10. Marvin, H. N., and Meyer, R. K., *ibid.,* 1941, v29, 965.

11. Kupperman, H. S., and Meyer, R. K., *Am. J. Physiol.,* 1945, v145, 181.

12. Biskind, M. S., and Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 176.

13. Moon, H. D., Simpson, M. E., Li, C. H., and Evans, H. M., *Cancer Research,* 1950, v10, 297.

14. Moon, H. D., Simpson, M. E., and Evans, H. M., *Science,* 1952, v116, 331.

15. Griffin, A. C., Rinfert, A. P., and Corsigilia, V. F., *Cancer Research,* 1953, v13, 77.

Received October 29, 1953. P.S.E.B.M., 1953, v84.