PROTOCOL III.
Fievre Boutonneuse Supernatant (4,000 R.P.M.)

Antigen dil.*	1/10	1/20	1/40	1/80	1/160	1/320	1/640	Serum control 1/10
Serum Dilutions—Fievre Boutonneuse Convalescent G.P. Pool.								
1/3	4	4	4	4	3	2	1	0
1/4	4	3	2	1	0	0	0	
1/6	1	0	0	0	0	0	0	
1/8	0	0	0	0	0	0	0	
Serum Dilutions—Rocky Mountain Spotted Fever G.P. Pool.								
1/3	4	4	4	4	4	4	4	0
1/4	4	4	4	4	3	3–	2	
1/6	4	3	1	0	0	0	0	
1/8	2	1	0	0	0	0	0	
Serum Dilutions—Normal G.P. Pool.								
1/3	2	1	0	0	0	0	0	0
1/4	0	0	0	0	0	0	0	
1/6	0	0	0	0	0	0	0	
1/8	0	0	0	0	0	0	0	

* Antigen anticomplementary at 1/2 dilution.

rickettsial antigens are used. This test may be employed with convalescent guinea pig or human serum thus providing a convenient laboratory test for diagnosing Fievre Boutonneuse. Since there is some cross fixation between Fievre Boutonneuse and Rocky Mountain spotted fever further evidence is presented to indicate that these diseases are antigenically related. Other members of the Spotted Fever Group are now being similarly studied.

The authors wish to thank Dr. R. R. Parker, Rocky Mountain Laboratory, for his kindness in giving us the Fievre Boutonneuse strain, Captain T. E. Woodward for obtaining the Fievre Boutonneuse human convalescent sera, Captain B. L. Bennett and Lt. A. B. Scoville, Jr., for maintaining the strain in guinea pigs and collecting the specimens of sera, and Corporal A. Rudolph for his technical assistance.

14507 P

Development of Tumors in the Rat Ovary After Transplantation into the Spleen.

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It has been shown by Golden and Sevringhaus[1] that rats in which the ovaries have been transplanted to the mesentery remain anestrous, owing to inactivation in the liver (previously reported by Zondek[2]) of the estrogen thus secreted into the portal circulation.

This phenomenon was more extensively studied by G. R. Biskind[3-6] using pellets of crystalline steroids implanted in another organ in the portal circulation, the spleen. Castrate

* Aided by grants from the Winthrop Chemical Company, the S.M.A. Corporation, and the United Hospital Fund of New York.

1 Golden, June B., and Sevringhaus, E. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 361.

2 Zondek, B., *Skandinav. Arch. f. Physiol.*, 1934, **70**, 133.

3 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

4 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 259.

5 Biskind, G. R. *Endocrinology*, 1941, **28**, 218.

6 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 452.

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rats on a complete diet with pellets of estrone or estradiol thus implanted likewise remained anestrous. During the course of an investigation on the effects of nutritional deficiency on the estrogen-inactivating function of the liver,[7-9] M. S. Biskind and Shelesnyak[10] demonstrated that animals with one ovary removed and the other transplanted to the spleen showed no estrual reactions so long as the diet was adequate to maintain the functional integrity of the liver.

This observation seemed to provide an excellent means of ascertaining the effect on the ovary of protracted stimulation by the pituitary, under conditions in which the latter organ is freed from the normal inhibitory action of the secreted estrogen.

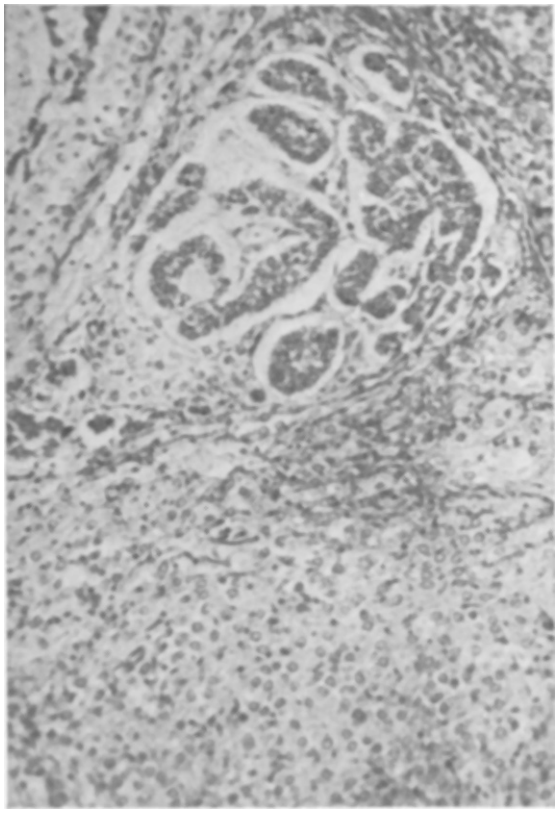

FIG. 1.
(S43-1089. 100× approximately). Small nest of granulosa cells situated in theca cell mass.

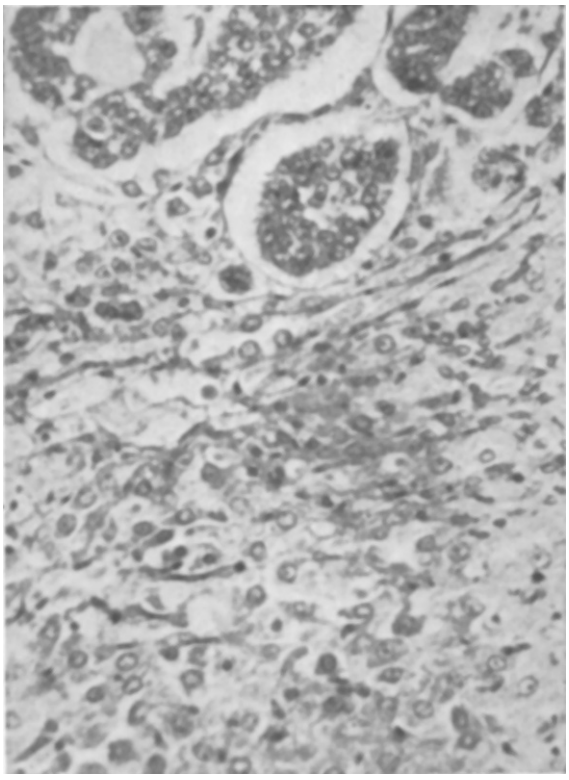

FIG. 2.
(S43-1089. 200× approximately.) Granulosa cells in ball-like clusters and forming small cysts; some theca cells are luteinized.

Accordingly, a series of adult female rats of the Sherman strain were castrated and one ovary was transplanted to the spleen.† After an interval of approximately 2 weeks, all the animals had daily vaginal smears to rule out those with vascularized adhesions that permitted ovarian estrogen to by-pass the portal circulation. Those showing estrous reactions were discarded. The others were maintained on a complete diet§ thereafter for approximately 11 months. At the end of this time

7 Biskind, M. S., and Biskind, G. R., *Science*, 1941, **94**, 462.

8 Biskind, M. S., and Biskind, G. R., *Endocrinology*, 1942, **31**, 109.

9 Biskind, M. S., and Biskind, G. R., *Endocrinology*, 1943, **32**, 97.

10 Biskind, M. S., and Shelesnyak, M. C., *Endocrinology*, 1942, **30**, 819.

† We are indebted to Dr. M. C. Shelesnyak for his assistance in performing the ovarian-splenic transplantations.

§ Rockland pellet diet with frequent supplements of lettuce, carrots, and meat.

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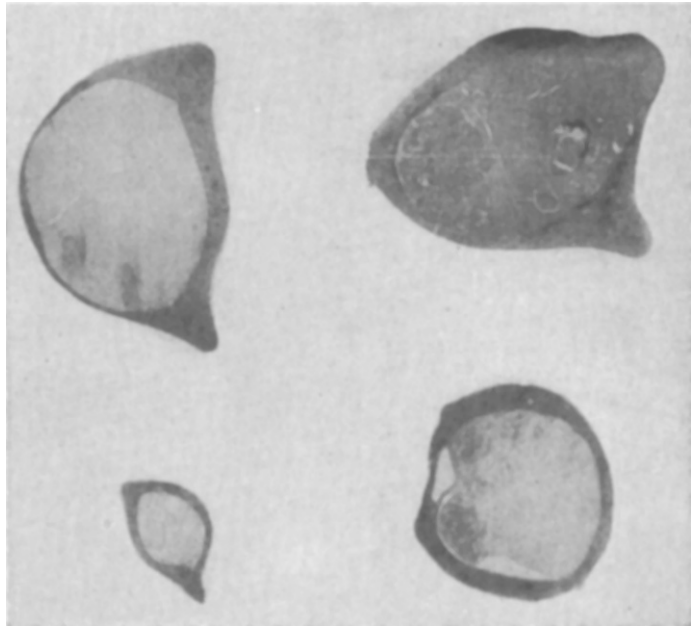

FIG. 3.
Three of the larger ovarian transplants described in the text, compared with a transplant of normal size[10] (lower left) all photographed to the same scale, approximately 3×. Figs. 1 and 2 are from the specimen in the lower right.

9 animals remained; they were again smeared daily over a period of 3 weeks to determine whether any estrogen was reaching the systemic circulation. Six remained completely anestrous; 3 had smears showing the occasional presence of a few epithelial or squamous cells but none of the latter at any time showed a complete estrous smear.

The animals were killed and the spleen containing the transplanted ovary, the vagina and uterus and the pituitary were preserved for histologic examination. In every case the ovarian mass in the spleen was much enlarged, the smallest measuring 7 x 5 x 3 mm, and the largest 15 x 13 x 10 mm. Normal ovaries removed from unoperated rats of the same strain and same age measured not larger than 5 x 4 x 3 mm.

On histologic examination all the ovarian transplants were found to be greatly hypertrophied and composed of masses of theca cells; all follicles had completely disappeared. In 3 of the transplants large tumors were found, apparently of the granulosa cell type, and in 2 others smaller nodules of the same cellular structure were found. With but 2 exceptions, the uterus and vagina showed castrate atrophy and the pituitaries were also of the castrate type. Two of the animals showed a slight estrogenic effect on the vagina, virtually none on the endometrium, and the pituitaries were of a modified castrate type. Thus for the most part, it appears that none of the estrogen secreted by the transplanted ovaries, or very little of it, was able to escape inactivation in the liver and the secretory activity of the pituitary was therefore entirely or largely unopposed by the inhibitory action of estrogen.

The granulosa cell tumors, as shown in the accompanying illustrations, are fairly sharply demarcated from the theca cell masses. They are made up of anastomosing cords and plugs and solid ball-like masses of small cells, indistinctly outlined. These cells have scant cytoplasm and a small round nucleus filled with fine chromatin granules. Mitotic figures are numerous in some regions, as many as 3 to 5

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in each high-dry field. In some areas the cells are arranged to form small cystic cavities containing a colloid-like substance, as in the "folliculoid" type of tumor. The vascular network is not prominent and there is very little discernible fibrous stroma. In many respects these resemble the ovarian tumors found by Brambell[11] and by Furth[12] in the ovaries of mice following roentgen irradiation.

[11] Brambell, F. W. R., Parkes, A. S., and Fielding, U., *Proc. Roy. Soc.*, Lond., Ser. B, 1937, **101**, 29.

[12] Furth, J., and Butterworth, J. S., *Am. J. Cancer*, 1936, **28**, 66.

14508

Development of Resistance to Pyrithiamine in Yeast and Some Observations on Its Nature.

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Since the development of resistance to various specific antimicrobial agents is a problem of much practical as well as theoretical interest, it has seemed advisable to attempt the production of variants of susceptible microorganisms which would be insusceptible or fast to pyrithiamine, and to study the means by which this fastness was achieved. It has recently been shown that pyrithiamine, the pyridine analog of thiamine, was a highly selective and effective inhibitor of the growth of microörganisms[1] and animals.[2] Only those organisms which were caused to grow more abundantly by the presence of thiamine were inhibited in growth by pyrithiamine. It was also shown[1] that the insusceptible species did not owe their resistance to the production of thiamine or other antagonist of pyrithiamine. If a fast strain of a susceptible species could be developed, it would be of interest to learn what alteration, if any, would occur in its thiamine requirements.

A pyrithiamine-fast strain of *Endomyces vernalis* was obtained in the following manner. Twelve successive transfers of the strain previously used were made in the synthetic medium,[1] to which had been added graded amounts of pyrithiamine ranging from 2 to 20 μg per cc. Consecutive transfers were made, beginning with the lowest concentration and terminating at the highest. After the yeast had been treated in this manner, its susceptibility to pyrithiamine was the same as that of the parent strain. It was then transferred 30 times in the synthetic medium plus 25 μg of pyrithiamine per cc. Care was taken to make each successive transplant as soon as growth was visible. Although in the first transfers growth appeared very slowly, after about the twentieth turbidity developed in 8 hours of incubation at 37°. The final culture obtained was labeled PF *Endomyces*.

PF *Endomyces* grew as well in a medium containing 25 μg of pyrithiamine per cc as it did in the absence of the compound. This was 25 times the amount of pyrithiamine which was sufficient to inhibit the growth of the parent strain half-maximally. However, the PF strain grew more slowly in the absence of pyrithiamine than did the parent strain. With the same size of inoculum, the PF strain required 40 hours to produce the same amount of growth made by the parent strain in 24 hours.

The PF strain had the same thiamine requirements as the parent strain. Thus it grew very poorly in the absence of thiamine, and was stimulated to grow either by thiamine or by its pyrimidine portion† alone. The quanti-

* With the technical assistance of J. Backstrom and J. Clifford.

[1] Woolley, D. W., and White, A. G. C., *J. Exp. Med.*, 1943, **78**, 489.

[2] Woolley, D. W., and White, A. G. C., *J. Biol. Chem.*, 1943, **149**, 285.

† Throughout this paper, the pyrimidine portion of thiamine refers to 2-methyl-4-amino-5-hydroxymethyl-pyrimidine.

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