agents. Since the combined treatment with sodium salicylate and calcium pantothenate proved effective experimentally, the clinical trial of the combination may be worthy of consideration.

Summary. ACTH, cortisone and sodium salicylate were capable of suppressing the phenomenon of local tissue reactivity. The dose of cortisone required for the inhibition was approximately 6 times greater than that of ACTH. Sodium salicylate completely inhibited the phenomenon in 17 out of 29 rabbits while in the remaining animals the reactions were strongly positive. Pantothenic acid, which had no effect upon the phenomenon by itself, enhanced significantly the suppressing effect of sodium salicylate.

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Demonstration of Increased Gonadotrophic Hormone Production in Castrated Mice with Intrasplenic Ovarian Grafts.* (18137)

O. J. MILLER AND CARROLL A. PFEIFFER.

From the Department of Anatomy, Yale University, New Haven.

Granulosa cell tumors, luteomas and mixed tumors occur in intrasplenic or intrapancreatic ovarian grafts in castrated rats(1) and mice(2). It has been demonstrated that the livers of animals of these species can inactivate estrogenic hormones(3-5) and that castrated rats or mice with intrasplenic ovarian grafts remain in diestrus after the first few weeks from the time of grafting(1,2). Therefore, it has been concluded that the pituitary glands of these animals are of the castrate type and produce greatly increased amounts of gonadotrophic hormones, with the resultant formation of ovarian tumors. In support of this concept, it has been shown (1,2,6) that the presence of an intact ovary, or of vascular adhesions between the graft and the body wall or the uterus, or the administration of estrogenic or androgenic hormones prevents the occurrence of ovarian tumors in intrasplenic ovarian grafts. No direct determination of the rate of production of gonadotrophic hormones in castrated animals with intrasplenic ovarian grafts has been reported. However, Jungck, Heller and Nelson(7) bioassayed the pituitary glands of castrated rats with intrasplenic ovarian grafts and found only a slight increase in gonadotrophic potency, with an equal increase in the rats with adhesions between the graft and the body wall. Their experiment was not critical, since the gonadotrophic content of the pituitary gland may not be proportional to the rate of release of gonadotrophins from it(8). The technic of parabiosis provides a method for assaying circulating gonadotrophins. The output of pituitary gonadotrophins is increased in castrated(9) and x-rayed(10) rats as de-

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1. Biskind, G. R. and Biskind, M. S., *Am. J. Clin. Path.*, 1949, v19, 501.

2. Li, M. H. and Gardner, W. U., *Cancer Res.*, 1947, v7, 549.

3. Heller, C. G., Heller, E. J., and Severinghaus, E. L., *Am. J. Physiol.*, 1939, v126, 530.

4. Talbot, N. B., *Endocrinology*, 1939, v25, 601.

5. Twombley, G. H. and Taylor, H. C. Jr., *Cancer Res.*, 1942, v2, 811.

6. Li, M. H. and Gardner, W. U., *Cancer Res.*, 1949, v9, 35.

7. Jungck, E. C., Heller, C. G., and Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 148.

8. Smith, P. E. 1939. Ch. XVI 931-965. *Sex and Internal Secretions,* Second Edition. Edited by Allen, Danforth and Doisy. Williams and Wilkins Baltimore.

9. Witschi, E., and Levine, W. T., PROC. SOC. EXP. BIOL. AND MED., 1934, v32, 101.

TABLE I. Ovarian, Uterine and Body Weights of Parabiotic Mice, One Member Castrated and Bearing an Intrasplenic Ovarian Graft.

Group	Animal No.	Days in parabiosis	Body wt (g)	Ovarian wt (mg)	% increase	t	P	Uterine wt Normal (mg)	Uterine wt Grafted (mg)	% increase Normal	% increase Grafted
1*	11	20	36.0	6.8				40.0	36.2		
	12	12	34.3	6.6				31.6	34.6		
	13	11	34.5	7.8				38.0	32.6		
	16	35	40.7	10.6				48.2	31.4		
	17	15	37.1	5.8				38.4	34.0		
	18	15	32.0	8.0				43.4	34.0		
	22	13	30.2	8.8				52.4	34.4		
	Avg	17	35.0	7.8	4	0.4	0.70	41.7	33.9	—30	—3
2†	27	11	35.6	6.6				32.8	34.6		
	28	9	40.8	13.8				114.0	31.0		
	31	12	36.2	8.2				40.6	34.6		
	33	13	38.8	13.4				52.6	24.4		
	34	12	38.5	10.6				125.0	36.6		
	35	12	39.9	11.4				62.4	25.6		
	38	10	37.6	11.2				86.0	25.8		
	Avg	11	38.2	10.7	43	2.5	0.04	73.3	30.4	23	8
3‡	25	5	32.5	5.6				64.6	29.2		
	29	4	33.7	7.6				53.4	20.2		
	30	4	35.3	8.2				52.0	24.2		
	32	2	35.2	8.8				73.2	45.2		
	36	7	36.8	7.4				54.6	46.4		
	Avg	4	34.7	7.5				59.6	33.0		

* Castration, intrasplenic transplantation and parabiosis performed at the same time.
† Castration and intrasplenic transplantation carried out 30 to 50 days prior to parabiosis.
‡ Same as Group 2, but autopsied from 2 to 7 days after parabiosis; served as controls.

termined by this technic. In the present experiment, parabiotic mice were used to demonstrate an increased gonadotrophic hormone output by the pituitary glands of castrated mice with intrasplenic ovarian grafts.

Material ond methods. Forty female mice of the Strong A strain were castrated at one to 2 months of age, and in each an ovary was transplanted into the spleen as an autologous or homologous graft. Each mouse was placed in parabiosis with a female litter mate; the operation was performed at the same time as grafting in 22 of the mice and 30 to 50 days later in 18 of the mice. The operation was performed as follows: A large segment of skin, extending from the base of the ear to the base of the tail, was removed from the right side of one and the left side of the other member of the pair. An incision was made through the abdominal musculature of each mouse, and the 4 cut muscle edges were sutured with a continuous silk suture. Anchoring sutures were placed through the adjacent scapulae and pelvic muscles. The skin edges were then approximated and held by a continuous silk suture. The parabiotic mice were kept in small cages in groups of from one to three pairs. Purina fox chow and water were available at all times. Some of the mice that received ovarian grafts from 30 to 50 days before being placed in parabiosis were killed or died from 2 to 7 days after parabiosis (Group 3) and served as controls. The remainder (Group 2), and the mice which received grafts at the same time parabiosis was performed (Group 1), died or were killed from 9 to 35 days after parabiosis. Twelve of the pairs pulled apart partially or completely; in 4 others there were vascular adhesions between the graft and the body wall. Mice with either of these conditions were discarded. Another 5 pairs were not autopsied because of advanced postmortem changes. The total body, ovarian and uterine weights of the remaining 19 pairs were determined at autopsy.

Results. It will be seen from Table I, which summarizes the results of these experi-

10. Levine, W. T., and Witschi, E., PROC. SOC. EXP. BIOL. AND MED., 1933, v30, 1152.

ments, that the average duration of parabiosis for the mice in which castration, intrasplenic transplantation and parabiosis were performed at one time (Group 1) was 17 days, with a range of from 11 to 35 days. The mice which were castrated and intrasplenic transplantation performed 30 to 50 days prior to parabiosis were divided into 2 groups in which the average duration of parabiosis was 11 days (Group 2) and 4 days (Group 3), with ranges of from 9 to 13 days and from 2 to 7 days, respectively. The body weights of the mouse pairs averaged 35.0, 38.2 and 34.7 g in the 3 groups. The average ovarian weight of the intact parabionts of the pairs in which castration and intrasplenic transplantation were carried out 30 to 50 days prior to parabiosis (Group 2) showed an increase of 43% over the controls (Group 3), while the pairs in which all operative procedures were performed at the same time (Group 1) showed only a 4% greater ovarian weight than the controls (Group 3). A comparison of the ovaries of the 2 groups (1 and 2) shows that castration and the presence of the graft for 30 to 50 days longer in the parabiont produces a 37% greater increase in the size of the ovaries. The same trend is evident in the pairs with the longest period of parabiosis in Group 1 (Table I); the ovarian weight of the mouse surviving in parabiosis for 35 days was 10.8 mg, in contrast to the 7.8 mg average for the group.

The average uterine weight of the intact parabionts in group 2 was 23% greater than in Group 3 which served as controls, and 73% greater than in Group 1 where all of the operations were performed at one time. The data for Group 1 is a measure of the very early changes. The average uterine weight of the castrated-grafted parabionts reflected the absence of estrogen stimulation and a certain amount of regression which was proportional to the length of time after castration and grafting. Thus, in mice in which castration and intrasplenic transplantation were done 30 to 50 days before parabiosis (Group 2) the average uterine weight was 8% less than in the controls (Group 3) and

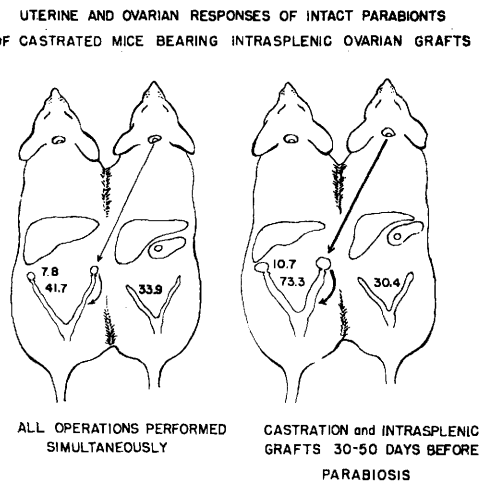

FIG. 1.

A schematic representation of the endocrine situation under the two conditions of parabiosis (Group 1 and 2). In each case the animal on the right is castrated and bears an ovarian graft in the spleen.

11% less than in Group 1 where all operations were performed at the same time. Fig. 1 shows a schematic comparison of Groups 1 and 2.

Discussion. The 43% increase in ovarian weight of the mice placed in parabiosis with animals that had been castrated and had received ovarian grafts in the spleen 30 to 50 days previously, and were autopsied 9 to 13 days later, is indicative of an increased rate of production of gonadotrophins by castrated mice with intrasplenic ovarian grafts. The lack of any significant increase in ovarian weight (4%), even during a slightly longer period of parabiosis in the pairs in which all the operative procedures were performed at the same time (Table I) does not conflict with this interpretation but rather indicates that there is no measurable increase in production of gonadotrophins during the first weeks after castration and grafting of an ovary into the spleen. The observations of Evans and Simpson(11) who noted a slow increase in pituitary gonadotrophic potency after castration in the rat might be taken to indicate that this increase could not be picked up by parabiotic experiments during the first

11. Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, v89, 371.

two weeks after castration. However, Biddulph, Meyer and Gumbreck(12) have shown that following simultaneous parabiosis and castration of one member of the pair the increased production of gonadotrophic hormone is easily measurable by the increased weight of the ovaries of the intact parabiont before the 11th day of parabiosis. If the mouse behaves the same as the rat, this would suggest that the increase in gonadotrophins in the castrate is somewhat inhibited by the presence of the ovarian graft in the spleen. With the increased stimulation of the ovaries of the intact member and the absence of the transfer of estrogen to the other member of a pair(13), the uterine responses are what would be expected.

The effects measured during the first two weeks of parabiosis may not be completely free from the influence of factors inherent in the operative procedure, although the findings of Biddulph, Meyer and Gumbreck(12) showed that fewer than 13% of the ovaries of intact female rats in parabiosis with castrates (both operations performed simultaneously) failed to hypertrophy by the 11th day of parabiosis. However, the absence of any significant ovarian hypertrophy in mice of Group 1, despite an average duration of 17 days in parabiosis, renders the 43% increase in ovarian weight that occurred in Group 2 in an average time of 11 days highly significant. It is realized that these experiments measure only the beginning of the gonad-hypophyseal imbalance present in a castrate bearing an ovarian graft in the spleen and that long term experiments in parabiosis are essential for its full elucidation.

Summary. Evidence of an increased rate of production of gonadotrophins in castrated mice with intrasplenic ovarian grafts has been obtained by means of parabiotic experiments. The ovaries of normal mice placed in parabiosis with female littermates that had been castrated and had an ovary grafted into the spleen from 30 to 50 days previously averaged 37% heavier than the corresponding ovaries when parabiosis was performed simultaneously with the other operations. The duration of parabiosis ranged from 9 to 13 and from 11 to 35 days, in the 2 groups, averaging 11 days in the former and 17 days in the latter.

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12. Biddulph, C., Meyer, R. K., and Grumbreck, L. C., *Endocrinology*, 1940, v26, 280.

13. Biddulph, C., Meyer, R. K. and Gumbreck, L. C., *J. Exp. Zool.*, 1941, v88, 17.

Inhibitory Effect of Cortisone on Anaphylaxis in the Mouse.* (18138)

CARL T. NELSON, CHARLES L. FOX, Jr., AND ELIZABETH B. FREEMAN.

From the Department of Dermatology and the Department of Bacteriology, College of Physicians and Surgeons, Columbia University. New York City.

Preliminary studies of electrolyte changes in the plasma and tissues of mice during anaphylaxis indicated that profound primary disturbances in the intracellular components of sensitized tissues follow the rapid union of antigen and antibody and that these changes, in turn, may cause the secondary hypovolemia and the shock-like circulatory collapse which follow(1).

The nature of these chemical alterations suggested to us that they might be inhibited, at least in part, by gluco-corticoid compounds. Should this be true, it would be of considerable importance. The question is of further interest inasmuch as it is now known that

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1. Fox, C. L., Jr. and Nelson, C. T., unpublished data.