sequently it was found that a 10% yield could be obtained in 65% ethanol–35% water, and the yield was increased to 15% in absolute ethanol and 19% in absolute methanol.

In a typical experiment, 60 mg of D-penicillamine hydrochloride and 66 mg of 2-benzyl-4-methoxymethylene-5(4)-oxazolone were condensed in 11.4 cc of pyridine and 0.6 cc of triethylamine at 75° for 15 min. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 10 cc of chloroform. The chloroform solution was extracted with 10 cc of cold 2 *M* pH 1.6 phosphate buffer and with 10 cc of 1.5 *M* pH 5.2 phosphate buffer, and was dried over anhydrous sodium sulfate. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 2.0 cc of 95% ethanol. Crystals of D-benzylpenillic acid formed over a period of 24 hrs, and during this time the absorption at 3,200 A gradually dropped. After 44 hrs, 14.5 mg of D-benzylpenillic acid were obtained.

The identity of the compound was established by its melting point, mixed melting point with authentic D-benzylpenillic acid, ultraviolet absorption spectrum, optical rotation, and conversion to dimethyl D-benzylpenillate (*1*).

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Atrophy of Ovaries Transplanted to the Spleen in Unilaterally Castrated Rats; Proliferative Changes Following Subsequent Removal of Intact Ovary[1]

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It has been shown that the transplantation of an ovary into the spleen of a castrate female rat leads to the development of a luteoma in that ovary after a period of approximately 5 months. If the transplant is permitted to grow for 10 months or more, a granulosa cell tumor supervenes (*1, 2*). The major factor concerned is the hormonal imbalance that results from inactivation by the liver of the estrogenic and other hormones elaborated by the transplanted ovary. The histogenesis of the tumor has been described (*2*). The important changes are the continuous formation of new primordial follicles, transformation of the follicles into corpora lutea, and growth of the corpora lutea with the formation of a large encapsulated luteoma in which granulosa cell nests arise (*2*). The appearance of these tumors after intrasplenic ovarian transplants in mice, guinea pigs, and rabbits has been described (*3, 5, 6, 7*). Confirmation of the growth in rats of the ovarian transplant (*4*) and formation of the tumors (*7*) has been published.

During the original experiments it had been found that if adhesions formed between the spleen and the systemic circulation so that the ovarian hormones by-passed the liver, the growth of the transplant was greatly retarded or would not take place. It was also noted that a pellet of estrogen placed in the subcutaneous tissue had the same effect on the transplanted ovary.

In the present experiments rats of the Long-Evans strain were used. In the first group there were 3 immature and 3 mature females. In the case of each animal one ovary was removed, cleaned of adherent structures, and placed in a pocket under the capsule of the spleen. The other ovary was not disturbed. One animal was sacrificed at each of the following days after transplantation: 24, 55, 90, 130, 150, and 246. In all animals the ovary *in situ* had undergone the usual compensatory hypertrophy that takes place after unilateral ovariectomy. The gross and histologic changes were the same in the immature and mature animals. The transplant in the spleen was much smaller than the original ovary; its position was indicated by the scar on the capsule of the spleen. Histologic preparations were made in the customary manner, and serial sections were studied. The changes were uniform except that in the youngest transplant, after 24 days, there were many small, active follicles with intact ova. No corpora lutea were present. The interstitial tissue was distorted by fibrous tissue. In all of the remaining animals the histologic structure was characterized by extremely few follicles that were relatively small in size. If ova remained, they appeared degenerated. There were no newly formed corpora lutea, and those that had been present in the mature ovaries had disappeared. The interstitial tissue was increased. In the animal examined after 246 days the degree of atrophy was severe; only two minute follicles were noted in a dense, fibrous interstitial tissue.

The second series of 4 immature animals was prepared by transplanting the left ovary into the spleen. After intervals of 63, 91, 91, and 91 days the hypertrophied right ovary was removed. After additional periods of 69, 97, 122, and 143 days the animals were sacrificed. Gross examination showed enlargement of the transplant. In the 3 oldest it was approximately 1 cm in diameter. Each uterus was atrophic, and the vaginal smears showed mainly leucocytes. Serial sections of the transplants were prepared. In the 69-day-old specimen the ovary was replaced by congeries of closely packed, large corpora lutea. Each corpus was quite distinctly outlined, and usually contained a small, central fibrous core. Scattered throughout were a few developing follicles. After 97 and 122 days the pattern was similar except that a rare fol-

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licle was cystic. After 143 days there was some loss of demarcation of the corpora lutea so that fusion of the corpora was suggested. Rarely was a fibrous core visible in these corpora. Primordial follicles were not evident. The picture suggested that the corpora were coalescing toward the formation of the luteoma.

Summary. An ovary placed in the spleen of an animal that has one normal ovary undergoes atrophy that starts as early as 24 days after transplantation. Severe atrophy is reached before 55 days, and subsequent changes are minor. The degree of atrophy is almost as intense as that noted in the hypophysectomized castrate animal with an ovarian transplant in the spleen (*2*). The transplanted ovary shows no evidence of any type of response to the hormonal influence that has stimulated the ovary that remained *in situ*. Removal of the normal ovary introduces the hormonal imbalance that was present in the original tumor animals. The atrophic ovarian parenchyma in the spleen in these, as well as in the hypophysectomized animals previously reported, retained its growth potentials and responded to the hormonal forces by assuming the changes that characterized the development of the luteoma. The action of the atrophic ovarian parenchyma is reminiscent of malignant cell deposits that may remain dormant or quiescent for long periods and then, for some unknown reason, suddenly undergo growth.

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Effect of Ryanodine on the Oxygen Consumption of *Periplaneta americana*

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Extracts of the plant *Ryania* have been found to possess insecticidal properties and are being investigated for physiological effects. Injections of the material produce paralysis and death in insects. The paralysis is of such nature that no responses can be obtained to mechanical or electrical stimuli, yet the oxygen consumption rises very noticeably. The oxygen uptake of the cockroach, *Periplaneta americana*, has been studied in the Fenn differential manometer before and after the injection of Ryanodine (concentrate of active principles of Ryanex ethanol solution, furnished by Merck & Co., Inc.).

Adult roaches of both sexes were used. Those used for controls were immobilized by confinement in a jacket consisting of a section of braided wire cable shielding of suitable size. These jackets were flexible enough to permit insertion of the animals without harm but rigid enough to prevent movement; they were also porous enough not to interfere with the movement of gases. The roaches were weighed, slipped into the jackets, and put into the manometer vessels, after which the O_2 uptake was measured for 2–2½ hrs at 25° C. They were then removed, and some were injected intrasternally with 0.05 ml of 0.01% Ryanodine, in an insect Ringer solution.

TABLE 1

O_2 Uptake and Percentage of Change Before and for the Period of 30 Min–2½ Hrs After the Injection of Ryanodine Extract or Ringer Solution

Ryanodine			Saline solution		
$mm^3/O_2/gm/min$		Change (%)	$mm^3/O_2/gm/min$		Change (%)
Before injection	After injection		Before injection	After injection	
5.14	16.41	203.3	5.97	6.45	8.0
3.91	12.09	209.2	4.84	6.91	42.8
1.96	7.89	302.6	4.34	6.72	54.8
6.71	20.34	203.1	2.27	5.35	135.7
6.04	18.12	200.0	6.34	10.34	63.1
9.25	14.52	57.0	6.06	15.55	156.6
6.02	17.76	195.0	10.12	11.87	17.3
5.43	15.20	179.9	7.12	8.56	20.2
5.00	14.69	193.8	5.90	5.26	−10.8
7.25	16.52	127.9	9.23	9.14	−1.0
9.14	13.75	50.4			
1.06	8.23	676.4			
4.61	14.15	206.9			
6.87	12.80	86.3			
5.16	13.12	154.3			
2.51	14.98	496.8			
5.88	17.00	189.1			
1.98	10.17	413.6			
2.94	8.57	191.5			
2.23	4.49	101.3			
Means 4.97	13.54	220.0	6.22	8.62	48.8

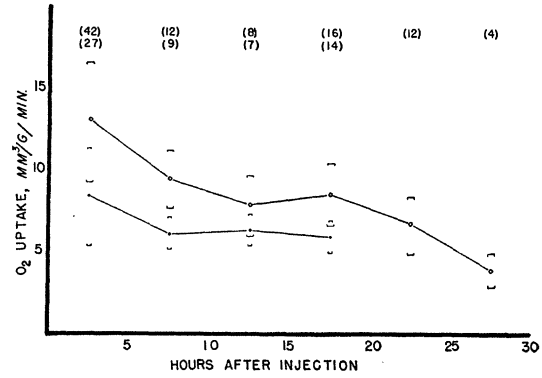

Fig. 1. Decrease in O_2 uptake, over a 30-hr period, of Ryanodine- and saline-injected roaches. Experimental series, ○; controls, ●. The points represent the mean values for 5-hr periods, and the figures in parentheses above give the number of readings represented by each point. The brackets above and below the points show the standard deviation of the mean value for each point.