Variants of *Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus subtilis* Requiring Streptomycin

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Recently, Miller and Bohnhoff (*2*) reported the isolation of a meningococcus variant which required streptomycin for reproduction *in vivo* and *in vitro*. This variant, called type B, appeared in maximum numbers when streptomycin was present in the medium in concentrations of 60–1,000 μg./ml. It produced a small, pearly-gray colony in the presence of low concentrations (60–100 μg./ml.) of the antibiotic, and a large, slightly yellowish colony with higher concentrations, as compared to the large, yellowish colony of the nonrequiring resistant strain, or type A.

anaerobic conditions. In addition, a small number of streptomycin-resistant organisms similar to the type A meningococcus reported above were noted. These produced a flat, grayish-white, spreading colony and were aerobic, nongranular rods.

The basal synthetic medium used in this work was similar to the one devised by Grossowicz (*1*) except for the exclusion of glucose, sodium thiosulfate, sodium d-glutamate, and thiamine. This basal medium contained only NH_4Cl as a nitrogen source and excluded all carbon-containing compounds, and these organisms could not grow until carbon compounds such as those indicated in Table 1 were added to the basal medium. Growth, which was measured by use of the Evelyn Photoelectric Colorimeter, was thus dependent upon the utilization of these added substances.

As indicated in the table, streptomycin stimulated the growth of all the organisms when glucose was present in the medium. Under similar conditions, only *Ps. aeruginosa* was stimulated when formate was present.

TABLE 1

GROWTH OF STREPTOMYCIN-RESISTANT STRAINS ON VARIOUS MEDIA WITH AND WITHOUT STREPTOMYCIN

1		2			3			4			5		
Medium		B.M. + Na formate			B.M. + glucose			B.M. + glucose + Na d-glutamate			B.M. + glucose + Na d-glutamate NH_4Cl		
Time (hrs.)		24	48	72	24	48	72	24	48	72	24	48	72
Strain	Streptomycin conc.												
E. coli													
Suscept.	0	97	95	96	70	60	52	*			*		
SM	0	91[2]	91[1]	91[2]	78[2]	58[2]	52[2]						
SM	250 μg./ml.	91[2]	91[2]	91[1]	66[2]	56	52[3]						
Ps. aeruginosa													
Suscept.	0	92	83	83	91	71	39	*			*		
SM	0	95	84	84	86[2]	53	30						
SM	500 μg /ml.	74	70	73	73[2]	43	36						
B. subtilis													
Suscept.	0	100	100	100	98	98	98	94	95	95	100	98[3]	96[1]
SM	0	100	100	100	97	97	94	92	94	95	99[2]	96	93[3]
SM	300 μg./ml.	97	97	97	75[3]	85	88	77[2]	75[2]	77[3]	79[2]	81[1]	83

Suscept. = susceptible strain; SM = streptomycin-resistant strain; B.M. = basal medium. * These organisms were not grown in these media.

We have recently made a similar observation in our laboratory. Strains of *E. coli, Ps. aeruginosa,* and *B. subtilis* that had developed resistance to streptomycin when grown on synthetic media yielded better and more rapid growth in the presence of the antibiotic. The variant strains had developed a resistance to 1,000, 8,000, and 1,000 μg./ml. of streptomycin, respectively, while growing on synthetic media.

A variant of *B. subtilis* was isolated which, on nutrient agar plates containing 150 and 300 μg./ml. of streptomycin, produced a grayish-translucent, spreading growth. This strain, which consisted of granular, rod-shaped organisms, grew only when streptomycin was present in the medium and only under

The growth of the *B. subtilis* strain in the presence of streptomycin and glucose reached its maximum level within 24 hours. However, when an additional carbon and nitrogen source was provided in the form of sodium d-glutamate, growth of *B. subtilis* was maintained for the entire period of observation at the maximum level it had attained with the glucose alone. The removal of some of the available nitrogen by the elimination of the NH_4Cl caused a slight decrease in that level. There was little or no growth of nonrequiring or susceptible organisms in any of the media used.

These results might be explained on the assumption that the *E. coli* and *Ps. aeruginosa* variants consisted of a mixture

of resistant and streptomycin-requiring strains, while the *B. subtilis* streptomycin strain consisted almost completely of a population requiring this antibiotic.

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Seasonal Variation in the Gonadotropic Potency of Dried Anterior Pituitaries of *Rana pipiens*[1]

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It is well known that the injection of anterior lobes of fresh pituitary glands may induce ovulation in a variety of animals. Among amphibia, Rugh (*3, 4, 5*) has shown that the gonad-stimulating potency is retained by the gland temporarily when preserved in distilled water and lower concentrations of alcohol, and may be maintained indefinitely when kept in 100 per cent alcohol.

TABLE 1

COMPARATIVE DOSES FOR FRESH AND DRIED FEMALE ANTERIOR PITUITARIES REQUIRED TO INDUCE AT LEAST 50 PER CENT OVULATION

	November	January	March
Rugh ('41) (fresh glands)	5	4	3
Hamburger ('42) (fresh glands)	5–6	3–5	2–3
Present data (dried glands)	4–7	4	2–3

In order to prevent loss of potency by injection in aqueous solution as well as contamination by traces of alcohol, a simple drying technique was devised by which anterior lobes of glands (*Rana pipiens*) were oven-dried at 94° C. for 48 hours and stored in desiccators until injected. A preliminary experiment conducted on 6 nonbreeding frogs collected in Wisconsin in November demonstrated that as few as 4 dried anterior pituitaries (from female donors) will induce ovulation, although as many as 7 or more may be needed on some frogs. Subsequent tests in January (25 frogs injected) and March (17 frogs injected) showed dried anterior pituitaries to be as effective as fresh glands in inducing ovulation, as determined by both the stripping method and direct observation of the ovaries. The effective doses for dried glands are compared with those given by Rugh (*7*) and Hamburger (*1*) for fresh glands, female donors being used in all cases (Table 1). As far as we are aware, there is no report in the literature concerning either the use of, or the doses necessary for, dried glands to induce ovulation in frogs. Rugh, however, states in a personal communication that the method has been used successfully in his laboratory.

The technique involving injection of dried anterior lobes has been applied to the problem of the determination of the relative gonadotropic potency of pituitary glands compared with the relative responsiveness of the gonad during various seasons of the year. The work of Rugh (*4, 5, 6*) indicates that the pituitary of the donor, as well as the gland of the recipient, may undergo seasonal changes. However, the variations in the gonadotropic activity of both are confused by seasonal changes in the responsiveness of the gonad. In some animals clear-cut seasonal changes in gonadotropic potency of the pituitary have been shown, as, for example, in the 13-lined ground squirrel, *Citellus tridecemlineatus*. Moore, *et al.* (*2*) were able to detect no gonad-stimulating hormone in the sexually inactive animals, but they found considerable gonadotropic substances on the approach of the reproductive season.

Glands removed from freshly caught frogs (*R. pipiens*) and dried in mid-January were compared in ovulation-inducing capacity with glands removed and dried in mid-March. The recipients, females in the prebreeding condition, were injected during the last week of March. All recipients, furthermore, were tested for ovulation by stripping and shown to be negative prior to injection. Control frogs did not ovulate throughout the duration of the experiment, terminated the last of April. Whereas an average of 4 January glands were necessary to cause at least 50 per cent ovulation (within one week after injection), 1–3 March glands induced ovulation in frogs in the same stage of breeding activity (Table 2). Although the number of experimental animals used was necessarily small, there is clearly a marked decrease in the number of donor glands required to induce ovulation between January and March. As all injections were made into March frogs, of comparable body length and breeding activity, it seems evident that the seasonal change is to be attributed primarily to ovulation-inducing capacity of the pituitary rather than to a change in the responsiveness of the gonad.

TABLE 2

OVULATION-INDUCING CAPACITY OF DRIED FEMALE ANTERIOR PITUITARIES FROM JANUARY AND MARCH FROGS TESTED BY RESPONSE OF MARCH FROGS

Gland source by date	No. glands per frog	No. frogs injected	No. showing at least 50% ovulation	% showing at least 50% ovulation
January	2	9	0	0
	3	10	3	30
	4	6	4	66
March	1	6	4	66
	2	6	4	66
	3	5	5	100

From these observations it is concluded that (1) desiccation does not alter the potency of the anterior pituitary, and (2) the gland undergoes seasonal variation increasing in gonadotropic potency between January and March.

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[1] We are indebted to Dr. Roberts Rugh of New York University for suggestions in the preparation of this manuscript.

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