

Presence of a Principle with Progesterone-like Activity Obtained from a Plant Source.

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The known biological incompleteness of the protein fraction of the bean has received considerable attention in interpretations of the malnutrition caused by a raw bean diet. Belák and Szathmáry,^{1, 2} and Zselyonka and Illényi³ were the first to point out that, together with this loss of weight, there was also observable an inhibition of the estrus of mice during the period of feeding with a diet containing 15% of raw bean. They isolated a globulin fraction from the bean flour, which they referred to as "phaseolin." Together with a loss of weight, the inhibition of the estrus of the mice could be observed when 5% of the basic food of the mice was replaced by this protein fraction. The fat-soluble fraction of the bean was found by the same authors to be ineffective. Selye and Collip,⁴ in replacing 30 to 50% of the basic food of the rat with bean flour, observed a certain atrophy of the ovaries and interpreted their findings as a consequence of a "malnutrition."

In our feeding experiments, first using rats as test animals, and following the course of the estrous cycles by means of the vaginal smear method of Long and Evans,⁵ we were able to establish the following:

1. Complete inhibition of the normal estrous cycle could be observed when 15% of the basic food was replaced by bean flour. Controls, on a diet without bean flour, failed to show any change in their regular cycles, whether that diet was offered to them (a) *ad libitum*, or (b) in an amount 15% less by weight than that regularly taken by these animals.

2. The pure globulin fraction of the bean, as prepared by Osborne,⁶ given in 5% by weight to the basic food did not inhibit the

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¹ Belák, S., and Szathmáry, J., 5th Congress of the Hungarian Physiol. Soc., Debrecen, June, 1935.

² Belák, S., and Szathmáry, J., *Biochem. Z.*, 1937, **291**, 259.

³ Zselyonka, L., and Illényi, A., *Biochem. Z.*, 1937, **291**, 263.

⁴ Selye, H., and Collip, J. B., *Endocrinology*, 1936, **20**, 667.

⁵ Long, J. A., and Evans, H., *Memoirs of the Univ. of California*, 1922, **6**.

estrus of the rats. The "phaseolin", however, as prepared by Zselyonka and Illényi,³ inhibited the estrus when added at the same level to the basic food. Osborne's method differs substantially from the other in that it involves a thorough extraction of the bean proteins with lipid solvents.

3. The raw bean flour, mixed in the diet to 15% inhibited the estrus of rats. However, the same bean flour was found ineffective in this sense even at a 20% level, after its thorough extraction in a Soxhlet apparatus, or after repeated thorough extraction at room temperature with fat solvents.

4. Furthermore, a crude oil extracted from the bean flour with fat solvents at room temperature in about 1.7% yield inhibited the estrus of the rats during the period of its subcutaneous administration. Controls, receiving the same amount of olive oil continued to show their regular estrus.

5. It was found that this estrus-inhibiting principle in the oil of the white bean, as extracted with lipid solvents, is directly antagonistic in its action on castrated female rats to the estrogenic hormone. One gamma estrogenic hormone (international standard ketoxyestryl), injected subcutaneously, first proved to be sufficient in our castrated rats to evoke estrus in about 100%. In cross-experiments the same amount of estrogen was found to be 50% less effective when the test groups were kept on a 15% by weight bean diet prior to, during, and after the administration of the estrogenic hormone.

The results of these experiments seemed to indicate that in the lipid-soluble fraction of the bean there is present a factor with a biological action similar to that of progesterone.

6. In order to examine this point further, the crude bean oil was tested in the Clauberg test, known as a most specific one for progesterone-like effects. We used the abbreviated form of the Clauberg test, as proposed by Butenandt, *et al.*⁷

With this test a positive progesterone-like effect of the bean oil could be repeatedly demonstrated. This is the first example of a substance from a plant source which exerts a progesterone-like effect.

This progesterone-like principle of the bean was found to be very sensitive to heat, oxidative agents and alkalies. Only extracts which took account of these properties retained their potency.

⁶ Osborne, T. B., and Strauss, E., *Abderhalden's Handbuch d. biol. Arbeitsmethoden*, Abt. I, Bd. 8, p. 425.

⁷ Butenandt, A., Westphal, U., and Holweg, W., *Z. physiol. Chem.*, 1934, **227**, 84.