

A Gonad-Stimulating Extract from Alfalfa Meal.*

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For reasons which can better be stated in a more complete report, it was thought highly probable that the pituitary hormone was not elaborated in the animal body from simple chemical substances, but on the contrary, was built up from complex precursors, or, taken in from the environment in a form not far removed from the hormone itself. If such hypothesis were correct, one should be able to isolate from foods these complex substances, or precursors, which when given to an animal in sufficient amounts, would prove to be gonad-stimulating. Of the foodstuffs considered, green plants seemed to be the most likely source of such material. Consequently, alfalfa was investigated from this standpoint and found to yield an extract capable of affecting the rabbit ovary in a fashion similar to that of the material found in pregnancy urine; *i. e.*, the production of corpora lutea and corpora hemorrhagica in unmated rabbits.

The extract is prepared by mixing 1 kilo of alfalfa meal with 10 liters of water and sufficient 3N alkali to bring the reaction to pH 9-10. After 4 hours the mixture is filtered by suction through a mat of moistened alfalfa meal, and the filtrate then subjected to the Katzman-Doisy benzoic acid method for the extraction of the active material from pregnancy urine.¹ The extracts prepared so far are highly toxic so that they can not be injected intravenously, and therefore cannot be satisfactorily assayed by the rabbit method. The material must be injected either intraperitoneally or subcutaneously, and in order to obtain a positive response it has been necessary to inject the equivalent of 300 to 500 gm. of dry alfalfa in 2 doses not more than 12 hours apart. From previous experience with injections of pregnancy urine by the intraperitoneal route, it is estimated that the yield from 1 kilo of dry material is probably more than 30 rat units and probably less than 100 rat units. The negative results obtained with the injections of inactive extracts, prepared in an identical manner, except for 24 hours (or more) initial exposure to the dilute alkali instead of 4 hours, together with the intraperitoneal

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¹ Katzman and Doisy, *J. B. C.*, 1932, **98**, 739.

TABLE I.
Results Obtained with Injections of Alfalfa Extracts.†
The dosage is expressed in terms of the equivalent of dry, crude alfalfa meal, one kilo being the equivalent of one entire extract.

Duration of alkaline extraction hr.	Extract	Dose in gm.	Ovarian response	
4	1	400	+	
	1	600	+	
	2	750	+	
	3	400	+	
	3	300	+	
	3	300	+	
	2	250	—	
	24	4	750	—
		5	500	—
5		500	—	
6		500	—	
6		400	—	
7		900	—	
8		800	—	
9		600	—	

† The successful extracts were prepared from 3 separate shipments of alfalfa which differed considerably in color, odor, texture, and protein content.

injections of a variety of materials, including male urine and sterol suspensions, serve adequately as controls.

It is apparent, then, that gonad-stimulating extracts can be prepared from alfalfa. The active material may, or may not, be related to the pituitary hormone, and these data may or may not have direct physiological significance, although they are in harmony with the working hypothesis which stimulated this work.

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Experimental Studies in So-called Agranulocytic Angina. I. Effects upon Leucocytes by Toxic Products of Bacteria from Stool of Human Case.

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The possible causal relationship of the intestinal bacterial flora in agranulocytic angina has not been investigated. We carried out bacteriological examinations of stool specimens of a human case

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