15138

No Androgen in Corn Pollen.*

LEWIS W. BUTZ AND RICHARD M. FRAPS.†

From the Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Md.

In connection with another investigation, we had the opportunity to apply to corn pollen a procedure found to be efficient for the extraction of androgens from animal material. Although no androgen was found upon assay of the extracts, it seems desirable to record the procedures employed. The main interest in the negative finding is that androgenic substances have not been reported to occur in plant materials, in contrast with the occurrence in plants of substances exhibiting estrogenic activity.

The pollen was collected by hand at Beltsville in August, 1944, chiefly from the hybrid US-13. Immediately after collection it was dried at 40°C, then kept in a covered glass jar at 0°C, and used 4 months later. Just before extraction the pollen was dried to constant weight at room temperature in vacuum over sulfuric acid.[1] The weight loss due to this last operation was 7.4%. Two portions, 139 g and 324 g, were taken. Each of these was worked up differently in order to insure adequate extraction of all components with properties similar to the known animal androgens and their inactive conjugates. The pollen was well ground under the solvents used for extraction.

Extraction. The smaller portion was thoroughly extracted by shaking with water at 40°C. The aqueous extract was fractionated by a procedure which has been found satisfactory for the investigation of urine.[2] This includes heating with barium chloride to liberate any androgen which may be present in inactive conjugated form. The water-insoluble residue, after thorough drying, was extracted with petroleum ether of boiling range 39-43°C. The solid residue, insoluble in petroleum ether, was subjected to the same treatment as the *n*-butanol extractives in the procedure of Talbot *et al.*[2] Hence two solutions in carbon tetrachloride and one in petroleum ether were prepared from the smaller portion of pollen.

The larger portion of pollen was thoroughly extracted in succession with petroleum ether and ethanol. The ethanol-insoluble solid was then fractionated in the same way as the smaller portion of whole pollen. This yielded finally 3 solutions in carbon tetrachloride and 2 in petroleum ether as shown in the accompanying scheme.

The carbon tetrachloride solutions were washed with water and dried with calcium chloride. The solvents were evaporated from the 8 solutions, and as much of the solvent-free extractives as possible (20 to 21 g) was dissolved in corn oil (Mazola), the last parts by heating on a steam-bath (80-90°C) for 30 minutes, to make 80 ml. This solution after cooling remained clear for several months; after 4 months the sediment which had deposited amounted to less than a gram.

Although any androgens similar to the known chemical types would be found in the corn oil solution, an attempt was made to assay also the extractives which did not dissolve since the quantity was large (not accurately determined, but about 20 g). This material was dissolved in ethanol to make 160 ml, but changes occurred in this solution, for after 3 days most of the material had precipitated out. The remaining solution, which contained 1.45 g of material not volatilized at 90°C and a slightly reduced pressure (this was determined on a small aliquot),

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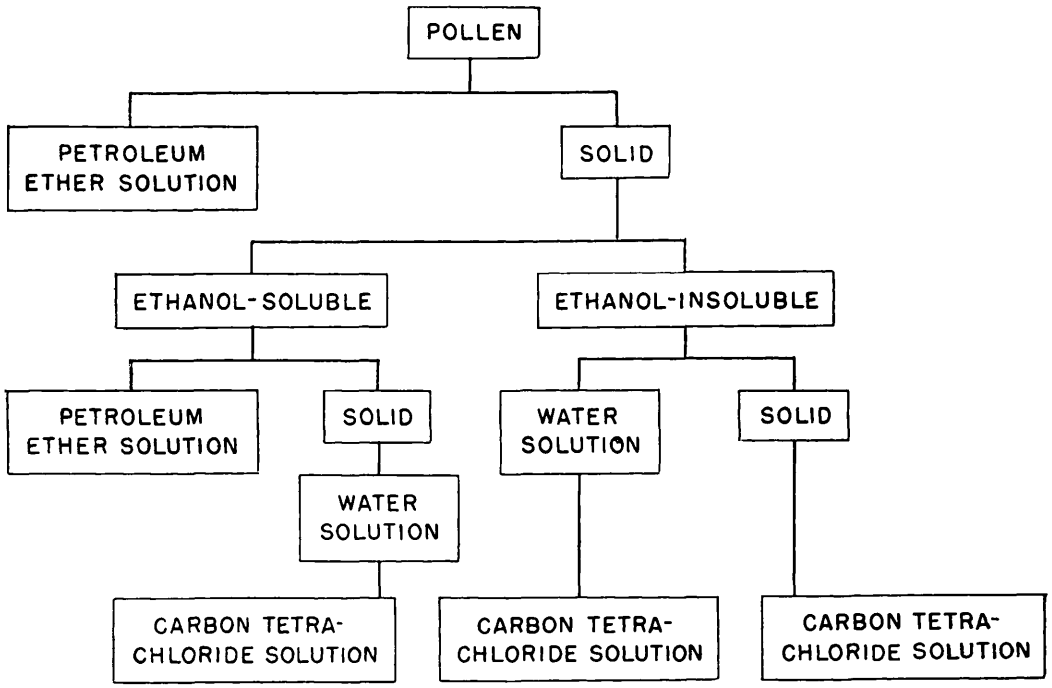
1 Anderson and Kulp, *J. Biol. Chem.*, 1922, **50**, 434.

2 Talbot, Ryan, and Wolfe, *J. Biol. Chem.*, 1943, **148**, 596.



was assayed both without further treatment and after removal of most of the ethanol.

Any water-soluble androgen not appearing in either the carbon tetrachloride or petroleum ether solutions obtained by the procedure described would have been missed in this work. Such androgens are unknown and therefore no methods for their bioassay have been developed.

Assays. The comb response of the Brown Leghorn capon was used for testing the extracts for androgenic activity. Both the corn oil and ethanol solutions were applied directly to the comb and injected subcutaneously.

Direct application to the comb was made with a syringe and needle, the material being spread as uniformly as possible over the entire surface of the comb. One to 3 applications were made daily, the number of applications depending upon the quantity administered and the consistency of the preparation.

The subcutaneously administered preparations were injected beneath the skin of breast areas, one-half the total quantity to each side. The material in ethanol was injected in the form of a reasonably homogeneous emulsion made by shaking the solution with 4 volumes of water just before injection.

TABLE I.
Effect of Extracts of Corn Pollen on the Comb of the Brown Leghorn Capon.

Test No.	Extract	Administration		Capons No.	Change in comb dimensions* mm
		Quantity	Route		
1	Ethanol sol.	0.2 ml	Direct	4	+0.50
2	" "	0.6 "	"	4	—2.50
3	" "	2.0† "	Subcutaneous	5	+0.25
4	" " conc.	0.2‡ "	Direct	3	—1.01
5	Corn oil sol.	0.6 "	"	4	—1.25
6	" " "	1.0 "	Subcutaneous	4	—0.25
—	Androsterone	0.2 mg	Intramuscular	9	+10.12

* Total change in length plus height found on days 6-8 for the tested preparations, day 6 for androsterone.

† 2.0 ml of the ethanol solution was mixed with 8.0 ml water daily just before injection.

‡ 0.2 ml of the concentrate is the equivalent of *ca.* 3.0 ml of the ethanol solution.

Control injections of androsterone were made intramuscularly into the breast in accordance with the method of Parkes and Emmens.[3]

Extracts under test were administered over 6 days, the androsterone over 5 days. Change in comb size was calculated as the sum of the changes in length and height occurring between the first day of treatment and the first or second day following the last administration.

The results of the assays are given in Table I. No androgenic response was observed after administration of either of the pollen extracts. The response of the capons to androsterone was almost exactly that previously observed by others.[3] It can be calculated from the figures presented that the smaller doses administered would have given a positive response if the corn pollen had contained 0.003% or more of an androgen as potent as androsterone and if 30% of such an androgen had been extracted by the procedure employed.

Summary. Extraction of corn pollen and assay of the extracts according to procedures commonly used on animal materials failed to yield any evidence of androgenic substances.

3 Parkes and Emmens, *Vitamins and Hormones*, Harris and Thimann, Editors, Academic Press, Inc., New York, 1944, **2**, 376.

15139

Incidence of the Eight RH Types Among 179 White Puerto Ricans.

MERCEDES VICENTE TORREGROSA. (Introduced by P. Morales Otero.)

From the Presbyterian Hospital, San Juan, Puerto Rico.

In the early work on the Rh factor, it was noticed that some bloods might be classified as Rh positive or Rh negative depending upon the human anti-Rh serum employed. It has been shown that human anti-Rh sera vary in their specificity, because they contain antibodies resulting from immunization to different Rh antigens. The anti-Rh sera that have thus far been found contain the following antibodies: anti-Rh_0, anti-Rh′, anti-Rh″, anti-Rh'_0, and anti-Rh''_0. The anti-Rh_0 sera correspond in specificity with anti-Rh sera obtained by immunizing animals with *Macacus rhesus* blood. The anti-Rh'_0 contain two antibodies, anti-Rh_0 and anti-Rh′; this holds true for anti-Rh''_0 containing antibody anti-Rh_0 and anti-Rh″.[1] Anti-Rh sera containing other antibody combinations have been found by British authors.[2]

Employing the 3 different varieties of human anti-Rh sera containing only one antibody, 8 Rh types have been defined by Wiener,[3] Rh_0, Rh_1, Rh_2, Rh_1Rh_2, Rh′, Rh″, Rh′Rh″, and Rh negative. Type Rh_1 may also be designated as Rh'_0, Type Rh_0 as Rh''_0, Type Rh_1Rh_2 as $Rh'_0Rh''_0$ to indicate the antibodies with which they react. (See Table I.)

There are some bloods giving weak reactions with some of these three varieties of anti-Rh sera; they have been classified as intermediate Rh types $Rh_1('')$, $Rh_2(')$, $Rh_0('')$, and $Rh_0(')$ (Wiener[4]). (Table II.) Murray, Race, and Taylor[5] have also described a rare agglutinogen, Rh_z.

The distribution of the various Rh blood types among white individuals in New York City and among Negroes can be seen in Table III.

With the data here presented, a study has been undertaken to determine the incidence

1 Wiener, A. S., *Am. J. Clin. Path.*, 1945, **15**, 106.

2 Stratton, T., *Nature*, 1944, **153**, 773.

3 Wiener, A. S., *Science*, 1944, **99**, 532.

4 Wiener, A. S., *Science*, 1944, **100**, 595.

5 Murray, J., Race, R. R., and Taylor, G. L., *Nature*, 1945, **155**, 112.