sided vessel, and examine its absorption spectrum by means of a simple hand spectroscope, using the same light source later to be used for the colorimeter. Dilute the solution, and inspect again. Note the region of the spectrum in which the maximum change of absorption occurs. Next examine, with the same light source, several light filters or solutions of various chemicals, until one is found which transmits light in the region of absorption by the above solution, but which absorbs as completely as possible all other light. Superimposition of the filter and the strong standard solution should shut out practically all visible rays. If the color filter finally adopted is of glass or other solid material, it can be placed over the eveniece of the colorimeter, cut to fit inside the ocular tube of the colorimeter, or placed over the source of light. If the color filter is a solution, it can be placed in a flatsided glass jar, as a museum jar, which is then placed in front of the colorimeter, or can be placed in accessory cups, of equal depth, one of which is placed

## PROBABILITY OF THE PRESENCE OF A SEX ANTAGONISTIC SUBSTANCE IN URINE

It has been frequently observed in this laboratory that certain extracts of normal, as well as of pathological urines, when injected into immature animals, inhibit the development of sexual organs. The results first obtained were not very uniform, as the treatment was never extended beyond a week. By extending the injections longer we obtained in a series of experiments typical sex organ inhibition. One of such experiments of 32 days' duration will be described here in detail.

Six male and six female rats of about the same initial weight were placed on an artificial food mixture consisting of casein, starch and salts with the daily addition of 0.5 gm dried yeast, 2 drops of codliver oil, 1 gm of dried milk and 400 mg of lard. The animals were divided into three groups, controls and two groups receiving daily subcutaneous injections of two different urine extracts, representing 250 cc of urine daily. The extracts were prepared in the following way.

Mixed male and female urines<sup>1</sup> (non-acidified) were extracted for 48 hours in a continuous extractor with ether. The ethereal extracts were evaporated and the residue extracted with as many cc of water, that one cc of extract represented one liter of the original urine used. The second extract was prepared from the under each colorimeter cup. A colored light source, as a neon lamp, may be used.

Where it is desired to block out the long end of the spectrum, a Bausch and Lomb blue hemoglobin filter can be used. An aqueous solution of picric acid blocks out the short end. These two superimposed allow practically no light to pass, except two faint bands in the green and in the yellow, respectively. A potassium chromate solution acts similarly to a picric acid solution. A solution of nickel sulphate blocks out both ends of the spectrum, and transmits the middle portion. If desired, a set of glass or gelatin filters may be purchased from an optical supply house, and will be found quite convenient. In many instances, however, they will be found not at all superior to the above-mentioned improvised filters, and ones similar to them, available in any laboratory.

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## SPECIAL ARTICLES

water-insoluble material, by dissolving the residue in a corresponding amount of peanut oil. The two extracts, prepared fresh every few days, showed the following general characteristics. The aqueous extracts were of a brown-yellow color and deposited on standing a brown oxidation pigment. They contained probably small amounts of the cortical suprarenal hormone and traces of female hormones. The oilsoluble extracts, on the other hand, contained no detectable amounts of andronin (male sex hormone), but relatively large amounts of theelin-like substances, which were, however, mostly insoluble in alkali. The animals were weighed every 4 days and the food intake determined every second day. The food intake was fair in all experimental groups and the growth of females was comparable in all the groups. These facts would practically eliminate the probability of toxic effects of the injections. After 32 injections the animals were sacrificed and the weight of testicles and seminal vesicles in the males, and of ovaries and uteri in females was determined in absolute figures, as well as per gm of body weight. The table below summarizes the experiment, the figures representing the average of two animals.

As contrasted with short experiments which show sometimes stimulation and sometimes inhibition of development of sex organs in males and a constant stimulation in females, the described typical experiment of longer duration leaves the males in the infantile stage, while a marked retardation is noticed in females. The difference in the degree of influence in the 2 sexes is tentatively explained by the compen-

<sup>&</sup>lt;sup>1</sup> Our thanks are due to the authorities and the head nurse of Hôpital Stell, Fondation Edward Tuck, in Rueil-Malmaison for supplying us with urines of patients for our investigations.

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Rats number	Weight		Per cent.	Food	Per gm	Gonads	Per gm	Sem. ves.	Per gm.	
	Initial	Final	incr.	int.	body wt.	in mg.	body wt.	in mg.	body wt.	
$\left.\begin{array}{c} 406\\ 407\end{array}\right\}$	49.5	153.5	210	584	2.8	4622.3	15.0	534 <b>.</b> 9	1.7	Controls
$\left.\begin{array}{c} 410\\ 411\end{array}\right\}$	53.5	172 <b>.</b> 5	222	626	2.6	4789.1	13.8	822.9	2.4	Aq. extr.
$\left.\begin{array}{c}414\\415\end{array}\right\}$	49.5	109.5	121	467	3.9	976.5	4.4	44.3	0.2	Oil-sol. extr.
FEMALES										
Rats number	Weight		Per cent.	Food	Per gm	Ovaries	Per gm.	Uteri	Per gm	a bi forman an dhanan an
	Initial	Final	iner.	int.	body wt.	in mg.	body wt.	in mg.	body wt.	
$\left. \begin{array}{c} 408\\ 409 \end{array} \right\}$	50.0	125	150	575	3.8	184.7	0.73	635.3	2.5	Controls
$\left. \begin{array}{c} 412 \\ 413 \end{array} \right\}$	48.5	119	145	531	3.8	232.3	0.97	623.0	2.6	Aq. extr.
$\left.\begin{array}{c} 416\\ 417\end{array}\right\}$	47.0	115.5	145	484	3.5	125.1	0.54	307.9	1.3	Oil-sol. extr.

MALES

satory effect of theelin-like substances present in relatively large amounts in the oil-soluble extracts.

The sex-inhibiting substance was found roughly in the same fraction which contains the male hormone and is present in normal urine. Control experiments of long duration injecting male and female hormones to their respective sexes, as well as treating male animals with female hormone and *vice versa* did not show any appreciable inhibitive effect on sex organs. Some of the inhibitive action of sex hormones, described in the literature, might be due to the presence in them of the substance described in this paper.

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## BERRIES RICH IN CALCIUM

HACKBERRIES<sup>1</sup> (*Celtis occidentalis* L.—Urticaceae) have long been used as food by various tribes of North American Indians. The Dakotas<sup>2</sup> pounded the fruit (including pits) and used them as a flavor for meat. The Pawnees pounded the fruit and mixed them with fat and parched corn. The Meskwaki In-

<sup>1</sup> Although popularly known as berries, hackberries are botanically drupes.

<sup>2</sup>Gilmore, 33rd Ann. Rep. Bureau Amer. Ethnol., p. 76, 1919.

dians<sup>3</sup> ground the fruit and made them into mush. Hackberries have a sweet pleasant taste with a flavor somewhat resembling that of figs.

The ripe fruit which was used in our investigation was collected during 1930 and 1931 in Nebraska and Kansas. Analysis of the fruit (pulp and pits were ground together) gave the following results (all figures refer to moisture-free fruit); reducing sugar (invert sugar) ranged from 32 to 36 per cent.; nonreducing sugars were absent; pentosans and hemicelluloses, 2-4 per cent.; crude fat (ether extract), 2.8 per cent.; crude protein, (N x 6.25), 11.6 per cent. While it is the usual practice to report separately the analyses of the edible portion of the fruit and the pit, in this article the analysis of the entire fruit is reported, first, because the Indians used the entire fruit, and secondly, because on account of the large pits and small pulpy part this is the only way to use the fruit economically.

The most interesting feature of this fruit is its mineral content. Analysis of several samples gave an average value of 29 per cent. ash, almost half of which (48 per cent.) was CaO, or 13.9 per cent. CaO in the moisture-free fruit. Evidence adduced below indicates strongly that the calcium is present in the fruit as calcium carbonate. In that case, 13.9 per cent. of CaO would correspond to 24.9 per cent.

<sup>8</sup> Smith, Bull. Publ. Museum, Milwaukee, p. 265, 1928.