quantitative and/or qualitative deficiencies in the diet. In our previous experiment on the influence of pregnancy and lactation[3] on caries activity, as well as in the present study, we used a sub-optimal diet in order to sharpen the nutritional deficiency of the group undergoing pregnancy, and thus obtain sharper differences of caries activity between the two groups in case of any relationship between dietary deficiencies and dental caries.

However, as it can be seen from the data presented, neither pregnancy and lactation[3] nor pregnancy alone influenced caries activity in any direction. These facts are in open contradiction with the alleged increase of caries activity as a consequence of dietary deficiencies during pregnancy.

Summary. The influence of pregnancy on caries activity has been studied in hamsters reared from weaning for 100 days on a sub-optimal diet, with their respective littermate controls. The results show that pregnancy does not influence caries activity in any direction.

Received May 26, 1949. P.S.E.B.M., 1949, **71**.

17164. Evidence for a Steroid Compound in Cane Juice Possessing Antistiffness Activity.

L. E. ROSS,* W. J. VAN WAGTENDONK, AND R. WULZEN.

From the Departments of Zoology, Indiana University, Bloomington, Ind., and Oregon State College, Corvallis.

The isolation from crude cane molasses and crude unheated cane juice of a fraction capable of alleviating an induced wrist stiffness in guinea pigs raised on a skim milk diet, was described by van Wagtendonk and Wulzen.[1] Later investigations by Oleson, *et al.*,[2] and Petering and coworkers[3] indicate that ergostanyl acetate and α-ergostanyl acetate are effective curative agents. We are therefore prompted to report that an active fraction of steroid nature has been isolated from the original cane juice fraction, and in our assay methods has proved to be a curative agent in dosages of at least 0.01 γ.

Experimental. A product obtained from the cane juice and melting at 81-82°[1] did not prove to be homogeneous when subjected to carbon-hydrogen analyses. It was therefore subjected to various procedures for further purification. Chromatic adsorption on columns of magnesium oxide or activated alumina failed to yield pure and homogeneous products. Repeated recrystallization from alcohol, followed by recrystallizations from acetone and cyclohexane yielded a compound melting at 164-166° that was homogeneous according to the carbon and hydrogen analyses and highly active in curing the induced stiffness in deficient guinea pigs.

a. Activity tests. The assay for activity was carried out according to the previously described methods.[1,4] The compound was dissolved in Wesson oil and administered orally to guinea pigs that had been kept on the deficient regime of skim milk powder to which the necessary vitamins and minerals had

TABLE I.

Therapeutic Test of High Melting Compound Carried Out on Guinea Pigs on Skim Milk Ration.

Level of assay in γ (5 times)	No. of animals	Cured	No cure
1	3	3	0
0.01	27	23	4

* Present address: Argonne National Laboratory, Chicago, Ill.

[1] van Wagtendonk, W. J., and Wulzen, R., *J. Biol. Chem.*, 1946, **164**, 597.

[2] Oleson, J. J., van Donk, E. C., Bernstein, S., Dorfman, L., and Subbarow, Y., *J. Biol. Chem.*, 1947, **171**, 1.

[3] Petering, H. G., Stubberfield, L., and Delor, R. A., *Arch. Biochem.*, 1948, **18**, 487.

[4] van Wagtendonk, W. J., and Wulzen, E., *Arch. Biochem.*, 1943, **1**, 373.

TABLE II.
Carbon and Hydrogen Analyses of the High Melting Compound.

Compound	C	H	Calculated for: $C_{27}H_{44}O$ C	H	$C_{28}H_{46}O$ C	H	$C_{30}H_{50}O$ C	H
High melting	84.35	11.73*						
	84.50	11.61*						
	83.43	11.38†						
High melting recovered from digitonin ppt.	84.02	11.55*						
Avg	84.06	11.57	84.31	11.53	84.35	11.63	84.44	11.81

* Analyses by Dr. A. J. Haagen Smit, California Institute of Technology.
† Analyses by Dr. H. L. Hunter, Eli Lilly and Company.

been added.[5] The results of the assay of the high melting compound (164-166°) are reported in Table I.

b. Chemical properties. The high melting compound gave a strong positive Liebermann-Burchard reaction. The compound could be precipitated with digitonin, and the original compound could be recovered from the digitonin precipitate by digestion with pyridine. The compound and its acetate showed an ultraviolet absorption spectrum (Fig. 1) with an inflection at 262-265 mu, and maxima at 272, 283, and 295.5 mu. The values of the extinction coefficient at the maxima were, for the high melting compound, respectively, 74.5, 77.1, and 45.5, and, for the acetate, respectively 35.6, 36.9, and 21.0. The carbon and hydrogen analyses of the high melting compound and of the product reisolated from the digitonin precipitation are given in Table II.

The acetate was prepared by refluxing 200 mg of the high melting compound for 30 minutes with 10 ml of acetic anhydride. The mixture was cooled and filtered. The crystals were washed, dried and recrystallized twice from 95% ethanol. The yield was 185 mg of colorless leaflets, melting at 141-143°, and having the following analysis:

Found:		C: 82.55	H: 11.02
		82.67	11.48
		81.63	11.03
	Avg	82.35	11.18
Calculated for:	$C_{30}H_{48}O_2$	81.76	10.98
	$C_{32}H_{52}O_2$	81.94	11.18

The acetate was saponified according to Sandquist and Gorton[6] with the results shown in Table III. The high melting compound was hydrogenated with platinum oxide as catalyst and acetic acid as solvent with the results reported in Table IV.

TABLE III.
Equivalent Weight Determination of the High Melting Compound.

Weight sample, mg	ml HCl, 0.100 N	Equivalent weight
46.5	1.10	447.2
63.3	1.39	455.4
55.2	1.24	445.2
Avg		449.3
Calculated for: $C_{29}H_{46}O_2$		426.6
$C_{30}H_{48}O_2$		440.69
$C_{32}H_{52}O_2$		468.74

TABLE IV.
Hydrogenation of High Melting Compound.

Compound	Catalyst, mg	Sample, mg	H_2 taken up, ml	Time of hydr.	Moles of H per mole compound
Cholesterol high melting compound	3.025	11.711	0.684	30 min.	1.00
	3.011	10.988	1.190	25 min.	1.93
	3.268	11.887	1.301	44 min.	1.89
	2.878	14.872	1.629	10 hr	1.93

[5] van Wagtendonk, D. J., and Wulzen, R., *Arch.* **155**, 337.

[6] Sandquist, H., and Gorton, J., *Ber.*, 1930, **63B**, 1935.

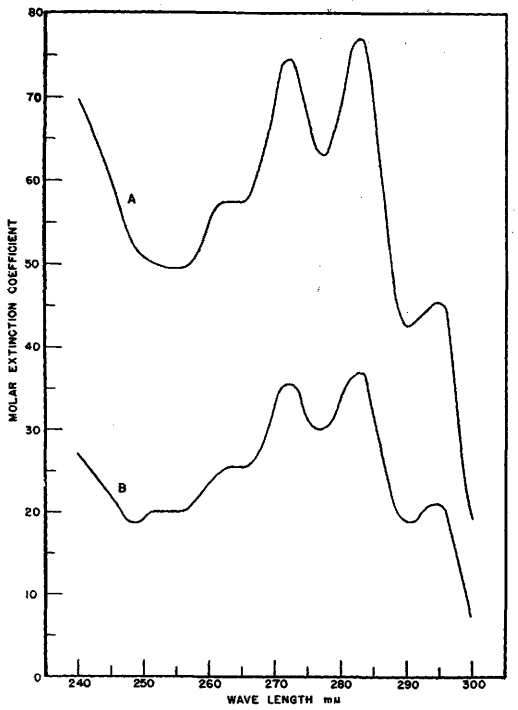

FIG. 1.

Ultraviolet adsorption of the high melting compound (curve A) and its acetate (curve B).

The acetate was hydrogenated in the same manner. The product of the hydrogenation was recovered from the solution and recrystallized once from 95% ethanol. The compound melted at 130.5-132.0° and had the following composition:

Found:		C: 80.52	H: 12.02
		80.71	12.03
	Avg	80.62	12.02
Calculated for:	$C_{29}H_{50}O_2$	80.87	11.70
	$C_{30}H_{52}O_2$	81.05	11.80
	$C_{32}H_{56}O_2$	81.29	11.94

Summary. A compound of steroid nature, having the probable formula of $C_{28}H_{46}O$, has been isolated from cane juice. This compound has therapeutic activity in alleviating an induced stiffness in guinea pigs. It has not been possible to further elucidate the structure of this compound, since not enough material was available at the time the project had to be discontinued, due to circumstances beyond control of the two senior authors. The isolations, however, tend to confirm the findings by Oleson *et al.* and Petering *et al.*, in that the antistiffness factor is steroid in nature.

Received May 20, 1949. P.S.E.B.M., 1949, **71**.

17165. Electron Microscope Studies of the Vesicle and Spinal Fluids from a Case of Herpes Zoster.*

ALFRED S. EVANS† AND JOSEPH L. MELNICK. (Introduced by F. G. Blake.)
(With the technical assistance of Ruth Peabody.)
From Section of Preventive Medicine, Yale University School of Medicine, New Haven, Conn.

The pathogenesis of herpes zoster has not been resolved. Even though recent work has suggested that the virus may multiply in the skin, the part played by the central nervous system in this infection is not clear. Like the virus of varicella, with which it is either closely related or identical, the virus of herpes zoster cannot readily be passed to laboratory animals, making work difficult in this disease. However, elementary bodies of varicella and herpes zoster have been described as occurring in vesicular fluids when such fluids have been examined in the optical microscope.[1-3] These bodies have been characterized more fully in the electron microscope by Ruska,[4] by Rake

* Aided by a grant from the Medical Fluid Research Fund of the Yale University School of Medicine.

† National Institutes of Health Postdoctorate Research Fellow.

Appreciation is expressed to Dr. John Craig for aid in obtaining specimens.

[1] Paschen, E., *Hyg. Rundschaw*, 1919, **29**, 269, 313.

[2] Aimes, C. R., *Lancet*, 1933, **224**, 1015.

[3] van Rooyen, C. E., and Illingsworth, R. S., *Brit. Med. J.*, 1944, **2**, 526.

[4] Ruska, H., *Scientia*, 1943, **37**, 16.