cies and of *Carica papaya* are to be attributed to the proteinases of these plants. However, there is some evidence on hand that certain other plant proteinases which are as proteolytically active as papain do not possess this peculiar ability to digest live worms.

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## CONVERSION OF ESTRADIOL TO ESTRONE IN VIVO[1,2]

WESTERFELD and Doisy measured the estrogenic activity of the phenolic fraction of the urine of monkeys injected with estradiol.[3] They found that 30 to 45 per cent. of the activity of the excreted estrogens was present in the ketonic fraction. After the administration of estradiol to estrous, hysterectomized estrous, or pregnant rabbits, Pincus showed the possible presence of estrone in the urine.[4] He was unable, however, to find any evidence for estrone in the urine of ovariectomized animals injected with estradiol. Neither the work of Westerfeld and Doisy nor that of Pincus was supported by chemical isolation and identification of metabolic products. This communication deals with the isolation and identification of estrone from the urine of long-time ovariectomized guinea pigs to which estradiol had been administered.

Ten mg of estradiol dipropionate in oil solution were administered by subcutaneous injection daily for 5 days to each of 5 adult guinea pigs which had been ovariectomized for at least one year. The urine was quantitatively collected during the injection period and for the following 5 days. After acidification with 10 per cent. of concentrated hydrochloric acid, the mixture was refluxed for 15 minutes and thoroughly extracted with benzene. That portion of the extract representing the phenolic compounds was separated by the Girard-Sandulesco reagent into ketonic and non-ketonic fractions.

The ketonic fraction contained more than 50,000 I. U. of estrogenic material when assayed by the vaginal smear test on the spayed adult mouse. The ketonic substances were subjected to high vacuum sublimation and the fraction subliming at 150° C. and 3 micra of mercury was collected. This semicrystalline material was crystallized from methanol and a crop of approximately 5 mg of crystals, m. p. 242–245° C., was recovered. After recrystallization from methanol, the melting-point was raised to 245–246° C. The melting-point of a mixture of this compound with an authentic sample of estrone (m. p. 255–258° C.) was 247–249° C. The benzoate melted at 211–214° C. When mixed with a sample of estrone benzoate (m. p. 215–217° C.), the melting-point was 211–214° C. All melting-points are uncorrected.

In a second experiment 50 mg of estradiol dipropionate were administered orally for 2 days to each of 5 adult guinea pigs spayed for at least one year. A procedure similar to that outlined above was used with the following modification: The phenolic compounds were fractionated between 0.1 N sodium hydroxide and 10 per cent. sodium hydroxide and the material soluble in the latter solvent separated into ketonic and non-ketonic portions by the Girard-Sandulesco reagent. About 12 mg of crystalline estrone, m. p. 256–257° C., were obtained from the ketonic fraction without resorting to high vacuum sublimation. It did not depress the melting-point of an authentic sample of estrone.

Thus it appears from this work that, at least in the guinea pig, estradiol may be converted to estrone even in the absence of the ovary. This and further work on the metabolism of the estrogenic hormones will be reported in detail.

We are indebted to Ciba Pharmaceutical Products, Inc., for the supply of estradiol dipropionate and estrone benzoate, and to the Schering Corporation for estrone.

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## TOMATO POMACE IN THE DIET

TOMATO pomace is the term applied to the dried residues that remain after the preparation of tomato juice. These residues contain the seeds, skin and some of the original pulp. The composition of the material[1] used in our studies was the following: Protein, 24 per cent.; ether soluble, 14 per cent.; fiber, 33 per cent.; ash, 4 per cent., and moisture, 7 per cent. An analysis for pectin by Z. I. Kertesz, of Geneva, N. Y., showed 3.8 per cent. of this substance.

Three properties of this tomato pomace attracted our attention while improved feeding mixtures were being developed for dogs, foxes and minks. A sample of tomato pomace was ground for a rat assay and for a carotene determination. After these were made the material was left in a mason jar without a rubber for a year in a warm laboratory. At the end of this time

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[1] This work was supported by grants from the Committee for Research in Problems of Sex, National Research Council: Grant administered by Dr. William C. Young; by the Rockefeller Foundation, and by the Fluid Research Fund of Yale University School of Medicine.

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[3] W. W. Westerfeld and E. A. Doisy, *Ann. Int. Med.*, 11: 267, 1937.

[4] G. Pincus, *Cold Spring Harbor Symposia on Quant. Biol.*, 5: 44, 1937.

[1] C. M. McCay, "The Nutritional Requirements of Dogs," p. 27. Ithaca, 1939.

it was assayed and the carotene determined again. Much to our surprise there was little decrease in the carotene content and in both cases the rat needed about 100 mg daily when this was the only source of vitamin A. This level, which is roughly 1 per cent. of the diet, permits a weekly gain of about 16 gm.

Furthermore, at the end of the year there was no evidence of rancidity either by taste or by the usual color tests such as the Kreis' one. Mattill and coworkers found antioxydants in tomato oil some time ago. There is a belief among some dog-feed manufacturers that dogs prefer freshly ground tomato pomace, but this has not been proved by rigid tests.

In the course of feeding diets that are relatively rich in carbohydrates to dogs, it is usual to observe periods of soft feces that may contain enough water at times to be considered a condition of diarrhea. While feeding a group of dogs a diet containing 5 per cent. of tomato pulp, the senior author observed unusual uniformity in fecal composition during a period of three months. When the tomato pulp was decreased to half this amount the uniformity in the fecal composition persisted. Subsequent observations with dogs have confirmed our earlier ones. Tomato pomace is being used in a number of dry dog feeds.

Nutritional studies of foxes and minks are being made by the junior author, and some of the experimental diets were causing loose feces that were entirely lacking in form. In some cases the looseness bordered on a diarrhea. It was found that by adding to the diet a quantity of ground, dried tomato pomace equal to 5 per cent. of the wet ration, the fecal form would change rapidly. In some instances the feces assumed good form and consistency within a day after the addition of the pomace to the diet and remained in good form as long as it was included. If the tomato pomace were excluded from the diet, the feces again returned to a loose condition. Tomato pomace contains carotenoids, and these were objectionable for diets planned to study vitamin A deficiency. For these diets the pomace was extracted for forty-eight hours with 95 per cent. ethyl alcohol to remove the carotenoids. This alcohol extraction did not affect the pomace so far as its desirable effects on the feces were concerned.

These observations have been reported at this time since they may have some use in both human and animal nutrition.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## THE PREPARATION OF PURIFIED HOUSE-DUST EXTRACTS

HOUSE dust is a frequent cause of allergic symptoms such as asthma and hay-fever. In common with other allergic extracts, house-dust extracts prepared by the usual method of simple extraction with aqueous extracting fluids contain a relatively large amount of material other than the allergically active substance. We are able to report at this time the preparation of highly purified, highly concentrated extracts which we have been able to show are allergically more active than house-dust extracts prepared by the methods in use previous to this work. House-dust extracts prepared by our technique uniformly produce strong positive scratch-test reactions in house-dust sensitive individuals.

For the purification of house-dust extracts, aqueous extracts of house dust were subjected to fractional precipitation by the addition of water-miscible organic solvents such as acetone, dioxane and isopropanol. The technique of the fractional precipitation may be described briefly as follows: The precipitate which formed when a small amount of the organic liquid was added to the original extract was removed, and then an additional amount of the organic liquid was added to the filtrate; this procedure was repeated to give progressively larger proportions of organic liquid with successive separations of insoluble fractions. In this manner the mixture of solids constituting the solute of the original house-dust extract was separated into a number of fractions.

Comparative skin tests[1] performed with solutions of the various fractions showed that the fractions precipitated by lower concentrations of the organic liquids, as well as those precipitated by concentrations of organic liquids above 75 per cent. were relatively allergically inert; the fraction precipitated by the intermediate concentrations of organic liquids possessed marked allergic activity.

This active fraction could be further purified by subjecting it to refractionation with the same or with a different organic liquid. Repeated refractionation of the purified active fraction resulted in no further separation of allergically inert material.

A greater degree of purification was obtained by subjecting the fractionated and refractionated extract to dialysis through number 1200 Cellophane membranes.

Extracts purified by fractionation with organic

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[1] C. H. Boatner, M. R. Pabst and B. G. Efron, "Analysis of Comparative Skin Tests." To be published.