SCIENCE

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<sup>1</sup> 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 <sup>2</sup> Agent in U. S. Bureaus of Biological Survey and Animal Industry.

<sup>1</sup>C. H. Boatner, M. R. Pabst and B. G. Efron, "Analysis of Comparative Skin Tests." To be published. liquids and dialysis contained a greater degree of skinreacting activity per unit of dissolved material than did the original extracts from which the purified extracts were processed.

The extract purified by fractionation and refractionation with organic liquids was further purified by treating it with high concentrations of soluble sulfates such as ammonium sulfate, sodium sulfate and zinc sulfate. The fraction precipitated from concentrated solutions of these sulfate salts contains a very high degree of specific allergic activity. It produces uniformly strong positive scratch-test reactions in housedust sensitive individuals in concentrations of 0.5 per cent.; specific intracutaneous tests are obtained with this extract in dilutions of 1/50,000 to 1/5,000,000.

Chemical analysis of all the fractions, the allergically active as well as the allergically inert, failed to show any significant difference in nitrogen or reducing sugar content.

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## A LABOR-SAVING TECHNIQUE FOR LEAF SAMPLES IN HISTOLOGICAL WORK

In the course of various studies on plant leaves it is often necessary to employ extensive sampling for histological material. The resulting collection of samples may come from various parts of the same leaf or from similar portions of different leaves. In either case the accurate recording of the source of each sample, as well as the maintenance of its identity throughout the stages in its preparation and storage before sectioning, is greatly facilitated by the use of India ink index numbers on the sample itself and upon the portion of leaf adjacent to it. The latter is then pressed, dried and kept as a record of the sample origin, or left in its original position on the plant when further samples are to be collected at a later stage in the development of the same leaf.

The general procedure is as follows: Duplicate numbers in India ink are put on the fresh leaves in the region from which the sample is to be taken. (Higgins waterproof black American India ink was used with satisfactory results.) A disc-shaped portion of the leaf including one of these numbers is punched out to furnish the sample, and the other number is left to record its source. A crow-quill pen is most satisfactory for producing small numbers without injury to the leaf tissues. With a clean pen no difficulty is encountered in numbering the leaves provided their surface is free of water. Care must be taken, however, that no pressure is applied by the pen to the tissues beneath, and that the numbers are dry before the samples are placed in the fixing fluid.

Following this technique the writer has fixed and preserved as many as fifteen leaf samples, each  $\frac{1}{4}$  inch in diameter, in a single 4-dram vial. The use of fourteen vials, index tabs and record entries in each group of fifteen samples was thus avoided. These sections can in turn be carried through dehydration and embedding as a unit, further avoiding fourteen out of every fifteen separate operations that would otherwise be necessary in these steps. In the process of embedding, the tissue samples are arranged in the warm paraffin so that they do not overlap one another, and so that the sides of the samples bearing the numbers face the lower side of the block, being thus legible through the thin paraffin layer. The resulting single paraffin block containing all the samples from a given leaf or plant has been found most convenient for storage and record-keeping purposes until sectioning is begun. Pressed and dried shoots bearing the leaves from which the samples were punched furnished a simple but excellent record of the sample origin.

The ink numbers are not dissolved or faded by chrom-acetic or by formalin-acetic-alcohol fixatives, or by any grade of alcohol in the ethyl or butyl alcohol series. The numbers withstand equally well the treatment of the samples with such clearing agents as dioxan and chloral hydrate. The numbered samples are clearly visible in the paraffin block permitting the ready location of any one of them desired for sectioning. The occasional necessity of sectioning directly through the numbers does not impose any restrictions on the use of the technique.

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