thymus that difficulty in differentiating the two tissues does not arise; often one can grossly distinguish the surface lymphoid follicles in the nodes.

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Plastic Chamber for Inert Atmospheric Work

The determination of physical constants of materials that are sensitive to the atmosphere requires expensive and elaborate equipment. We have designed an inexpensive dry-box that can be used to determine the refractive indices of materials that are oxidized by atmospheric oxygen. To make these determinations, an Abbé refractometer must be enclosed in an atmosphere-tight container having the visibility necessary for efficient operation of the instrument. Commercially available dry-boxes are unsatisfactory for this specialized purpose because of size, cost, visibility, and weight. Polystyrene was used to construct the dry-box shown in Fig. 1 because it would provide the desired properties of the container and retain ease of construction.

A cement made of polystyrene dissolved in trichloroethylene was used to join the component parts, ½ in. polystyrene base, 12.5 × 18 in., ¼ in., polystyrene sides, 9 × 12 in. and 9 × 18 in., and 0.01 in. polystyrene top. The water, electric, and nitrogen inlets were sealed with the same cement. The glove ports were made by bending with heat a 2 × 17.5 in. strip of ¼-in. polystyrene around a pipe. To make bends in the thin polystyrene to form the top, the seams were moistened with trichloroethylene to soften the plastic. Any leaks that may be detected are readily sealed by application of the cement.

The use of this box was found to be quite satisfactory. The refractometer scale could be read with ease through the top, and the box was sufficiently light to

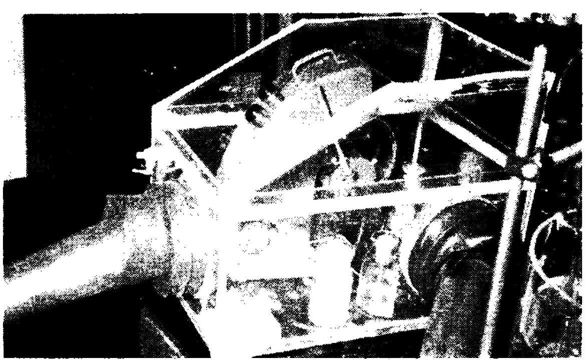

Fig. 1. An apparatus for determining refractive indices in an oxygen-free atmosphere.

be moved even with the refractometer inside. Some leakage occurred at the glove ports, but this was not critical, if the pressure inside the box was kept greater than atmospheric pressure. A sheet of aluminum foil on the bottom of the box prevented spilled organic liquids from softening the plastic. For the removal of traces of oxygen remaining after sweeping the dry-box with an inert gas, a weighing bottle containing a glass-wool wick saturated with tri-n-butylborane, a substance that is readily oxidized, was opened. Materials were introduced into and removed from the dry-box through the glove ports.

With slight adaptations the box could be used for containing other pieces of apparatus or for work in an anhydrous atmosphere.

I wish to express appreciation for the technical assistance of Helmut Haendler and Walter Eldredge and for advice received from the Plax Corporation of Hartford, Connecticut.

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24 September 1954.

On Column Chromatography of Sugars

The use of carbon (*1*) and cellulose (*2*) columns has become widespread for the separation of carbohydrates of comparatively low molecular weight. It is therefore desirable to point out several factors of importance in the general use of such columns.

When using carbon columns made of any of a variety of charcoals, we have found it expedient to give the column a preliminary wash with dilute hydrochloric acid solution in order to assure the removal of basic ash, which might otherwise cause some isomerization of the sugars applied later. A 1 percent hydrochloric acid solution is sufficient. The acid is then removed from the column by washing with distilled water. Celite (*3*) is usually mixed with finely ground charcoals (*4*) to increase flow rate. However, celite sometimes dissolves in the developing solutions and is obtained as a flocculent precipitate in the concentrated effluence. Celite can be removed from the concentrated effluence by filtration through a bacterial filter or by evaporation of the solution to dryness and redissolution of the carbohydrate in water. To avoid this inconvenience we often use columns composed entirely of charcoal. The charcoal selected is that which passes a 40- or 60-mesh screen but is retained on an 80-mesh screen. This produces a column composed entirely of charcoal and consequently increases sorptive capacity of the column.

Often in the use of cellulose columns, carbohydrates other than those placed on the columns are observed in the eluates. These extraneous carbohydrates arise from the cellulose or disintegrated filter paper employed to pack the column. The cellulose used is, of course, not chemically pure but represents a purified pulp that still contains a small amount of hemicellulose. These extraneous carbohydrate substances are most often observed in the initial column effluent, particularly if water is passed through the column. Extraneous carbohydrates are also sometimes encountered in later effluents when liquids other than pure water are used. To reduce the amounts of these extraneous substances, it is suggested that cellulose columns be given a preliminary washing with water before use. While we presently know of no way to prevent completely the elution of extraneous substances from cellulose columns, we wish to call attention to their presence.

In operating chromatographic columns, particularly those of considerable length, it is desirable to apply positive pressure at the head rather than suction on the effluent. Application of vacuum often causes the lower portion of the column to be partially freed of solvent with consequent poor separations in these regions.

There seem to be two schools of thought concerning whether columns should be packed dry or by the slurry method. In our experience either method is good when properly handled. In packing by the slurry method care must be taken to use a thick slurry and thus prevent fractional separation of particle size or, in the case of a carbon-celite mixture, separations of the two components.

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4. Such as Darco G-60, a product of Darco Corp., New York, N.Y.

14 October 1954.

Experimental Arteriosclerosis; Sulfur or Choline Deficiency?

A description of a kind of vascular abnormality produced in young rats by dietary means has been described by Wilgram, Hartroft, and Best (*1*). The authors attribute these arterial effects to a deficiency of choline, although the diets employed were profoundly deficient in essential organic sulfur compounds as well. Mortalities of 50 and 75 percent of the experimental groups in the 28-day periods of observation attest to the rigors of the diet. These experiments should be considered in relation to the chronicity of arteriosclerosis toward which all such experimentation is ultimately directed.

In their discussion the authors mistakenly observe, when referring to our work with experimental atherosclerosis in *Cebus* monkeys, that we have not specifically investigated the effects of choline deficiency on the vessels of monkeys. We have, in fact, done this and our observations were described (*2*). It was found that choline deficiency effectively *prevented* sufficient hypercholesterolemia to produce atherosclerosis in the monkeys. I believe that is because the animals became ill and refused to eat.

Our most atherogenic diets regularly contain 0.5 percent choline. We also observed (*2*) that this disease in monkeys is either prevented or cured with *l*-cystine and this effect has since been extended to several congeners of cystine with similar effects (*3*). It is perhaps of interest to those who press for the importance of choline in a variety of ailments that our discovery of the relationship of sulfur metabolism to atherosclerosis in monkeys resulted from the great difficulty we experienced in producing evidences of choline deficiency in this primate species.

Finally, Wilgram *et al.* propose that the much abused term *lipotropic* be extended to still another poorly understood phenomenon: the prevention of the accumulation of lipids in blood vessel walls. This redefinition of the term can scarcely do more than add to the existing confusion. The variety of meanings of *lipotropic* both in respect to anatomical structures involved and to the methods known or presumed to have induced the lipid deposition will require a short qualifying paragraph for each context in which the term is used.

It is well known that if a hungry cat consumes a saucer of cream, it will shortly show an accumulation of lipids both in its intestinal mucosa and in its liver. Are we to apply the term *lipotropic* to a milkman who does not come, to a more aggressive cat, or even to the large mouse that may have already satiated the cat? This proposal surely defeats the purposes of language.

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5 August 1954.

We have had little experience with *Cebus* monkeys but are now starting an investigation of the role of various dietary factors in the production of atheromatous lesions in small primates. George V. Mann's previous studies have been of great help to us in planning this work. His present letter has raised several interesting points on which we would like to comment.

In the first place, Mann is discussing atherosclerotic lesions seen in monkeys that have been consuming diets high in cholesterol; we have described aortic medial sclerosis produced in rats by diets low in choline and essentially free from cholesterol.

He states that we attribute arterial lesions in choline-deficient rats ". . . to a deficiency of choline, although the diets employed were profoundly deficient