CORN AS AN ETIOLOGICAL FACTOR IN THE PRODUCTION OF A NICOTINIC ACID DEFICIENCY IN THE RAT[1,2]

THE consumption of corn has been associated with pellagra since the time of Casal in 1735. Handler,[3] working with dogs, has indicated that corn may be a causative agent in pellagra, and we[4] have found the nicotinic requirement of dogs to be markedly increased when corn grits are added to synthetic rations. With rats on rations containing corn, Frost and Elvehjem[5] found a very slight growth increase when nicotinic acid was fed, but they recognized that the rations used were deficient in other factors, thus complicating their results.

By using a synthetic ration containing sucrose 78, casein 15, corn oil 3, salts IV 4 and cystine 0.15 parts so prepared as to be essentially free of nicotinic acid (< 0.01 mgs per 100 gm) but adequate with respect to all other known fat and water-soluble vitamins except "folic acid" and p-aminobenzoic acid, we have produced a pronounced growth retardation in the rat by adding 40 per cent. yellow corn, white corn or corn grits at the expense of the entire ration. The addition of nicotinic acid at levels of from 0.5 to 1 mg per 100 gm of the corn-supplemented ration completely counteracts the growth-depressing action of corn. A specially prepared nicotinic acid-low norite eluate from solubilized liver extract fed at a level equivalent to 11.5γ of B_c (*S. lactis* assay) per 100 gm of ration failed to counteract the deleterious effect of corn. These data are summarized in Table 1.

In addition we have found that the kind of carbohydrate and the level of casein modify the extent of the untoward effect of corn. With glucose as the carbohydrate or when the level of casein (nicotinic acid content < 0.005 mgs per 100 gm) was raised to 20 per cent. the growth depression caused by corn was diminished. In all cases, however, the addition of nicotinic acid resulted in growth stimulation.

TABLE 1

GROWTH-RETARDING EFFECT OF YELLOW CORN AND CORN GRITS AND THE COUNTERACTION WITH NICOTINIC ACID

Rations used			Average weight gain in 4 weeks; 3 animals per group
Experiment A			grams/week
Sucrose basal			30 (22–38)
"	"	+ 1 mg per cent. nicotinic acid	28 (21–33)
"	"	+ 40 per cent. yellow corn	13 (9–16)
"	"	+ 40 per cent. yellow corn + 1 mg per cent. nicotinic acid	33 (30–36)
Experiment B			
Sucrose basal		(same as in experiment A)	25 (20–29)
"	"	+ 40 per cent. unenriched corn grits	4 (2– 5)
"	"	+ 40 per cent. unenriched corn grits + 1 mg per cent. nicotinic acid	27 (21–29)
"	"	+ 40 per cent. unenriched corn grits + "folic acid" prep.* ≡ to 1 per cent. solubilized liver extract or 11.5γ B_c (*S. lactis*) per 100 gm	5 (4– 6)

* The "folic acid" preparation was so prepared as to retain most of the B_{10} and B_{11} activity.

Unenriched corn grits which contain 0.7 to 1.0 mg of nicotinic acid per 100 gm produce more profound growth depression than does yellow corn meal, which contains about 2.0 mgs per 100 gm. It is interesting to note that polished rice or rolled oats, both of which contain significantly less nicotinic acid than whole yellow corn, produce no growth depression when fed under identical conditions. One sample of white corn has been tested and was more effective in retarding growth than was yellow corn.

Evidence at hand indicates that milk, although very low in nicotinic acid (*ca.* 0.8 mg per cc), is active in counteracting the growth depression caused by corn.

Complete details of this work and the additional investigations which are in progress will be reported at an early date.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHODS FOR DETERMINING REFRACTIVE INDICES IN POLARIZED LIGHT MICROSCOPY

A SIMPLE but effective method for determining refractive indices in polarized light microscopy has been used successfully by students in these laboratories for the past three years. The main advantages of the method are that it is rapid and requires no extensive knowledge of geometrical or optical crystallography. It is of particular interest, therefore, to the chemist who has occasional need for microscopical observations under polarized light, but can not afford the time necessary to master the crystallography and physical optics required by the more extensive microscopical procedures.

[1] From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

[2] Published with the approval of the director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Dairy Council, Chicago, Illinois.

[3] P. Handler, *Proc. Soc. Exp. Biol. and Med.*, 52: 263, 1943.

[4] W. A. Krehl, L. J. Teply and C. A. Elvehjem. Unpublished work.

[5] D. V. Frost and C. A. Elvehjem, *Jour. Biol. Chem.*, 128: 23, 1939.

The determination of index of refraction for isotropic substances is carried out by the usual Becke line or half-shadow methods. In the case of anisotropic crystals, however, the customary order of procedure is reversed. A particle is first located which shows an interference figure under conoscopic observation, and from this the optical class and optic sign are determined. Next, refractive indices are determined on crystals showing the maximum flash of color during rotation between crossed nicols. By rotating such crystals to extinction and removing the analyzing prism, either the high index or low index will be shown. Further rotation of 90° to the second extinction position gives the remaining index. The indices so determined are identified from the previously determined optic sign.

If the crystals are uniaxial positive the lowest index found will closely approximate the value for omega; if the crystal is negative, the omega index will be very near the highest value found. In the case of biaxial crystals, the low value represents alpha and the high value approximates gamma. For biaxial positive crystals, beta will be nearer alpha than gamma, while in negative crystals it will be nearer gamma. The value for beta can usually be estimated with a fair degree of accuracy from these relationships. If desired, the value for beta can be checked on a crystal which shows an interference figure having the optic normal in the north-south position. A check on the omega index of uniaxial crystals can be made on crystals showing centered interference figures.

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ARRANGEMENT FOR DRYING PROTEINS FROM THE FROZEN STATE

THE drying of proteins and similar products from the frozen state is now a daily requirement in many laboratories, and any arrangement which disposes of the chore efficiently can be used to good advantage.

For more than a year the apparatus sketched in Fig. 1 has been used effectively. One can dry any volume of material up to 200 cc, and have it in any appropriate container such as beaker, test-tube, centrifuge tube, Erlenmeyer flask, evaporating dish, ampoule and the like. As a result wasteful transfers of highly valuable materials can many times be avoided. The specifications for the apparatus can vary considerably and my description covers only one way of putting the general idea into operation.

Altogether there are three units. One of these (A) is a well-insulated sheet metal box $12 \times 12 \times 14$ inches outside diameter. The box is provided with a lid, and is filled with dry ice to supply refrigeration. Through the box there is a built-in round channel about $3\frac{1}{4}$ inches in diameter. The walls of the latter are of sheet metal similar to the metal walls of the refrigerator box itself.

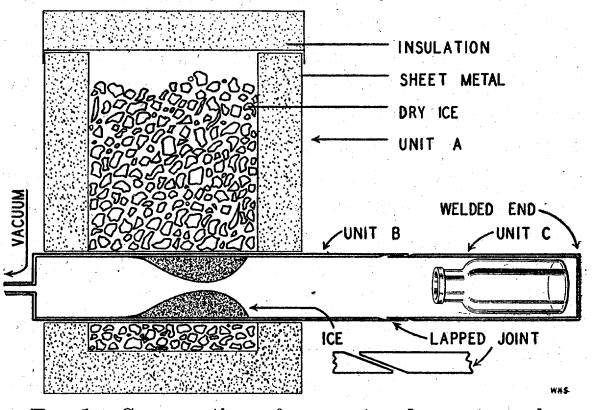

FIG. 1. Cross section of apparatus drawn to scale.

Units B and C are made from the same stock of 3-inch malleable black iron pipe or brass tubing, and are connected by a lapped joint. This joint must be machined accurately, lapped carefully and handled as one would a ground glass joint or the ground glass surfaces on desiccators. When unit B is in the round box channel it is cold and becomes a condenser, and unit C, which is warm, contains the material to be dried. During the short time required for establishing a vacuum unit C must be cold. That detail can be handled either by precooling or by pushing both B and C to the left until C is in the cold channel of the box. During the drying operation it is well to direct a fan at unit C.

If 100 cc of water are shell frozen in a large centrifuge bottle it is best to pack the box full of dry ice and conduct the run as an overnight operation. That practice can be recommended for most of the average work.

With the use of ordinary ingenuity the apparatus can be handled and modified to achieve special objectives. For instance, heat can be applied to C for the purpose of removing the last traces of moisture toward the end of a drying operation. For that purpose there are suitable heating devices available. Or one may wish to seal material under vacuum. To do that holes are drilled in unit C, suitable taps are provided for the purpose of permitting rubber connections to ampoules, etc. Another degree of freedom can be exploited by completely setting aside unit C and substituting a balloon flask. Connections are then made with the use of a rubber stopper. The flask is roughly covered with wire screen for protection against possible breakage.

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