

an average value for the amount of blood consumed per day per recovered worm. Thus:

Let R = total radioactivity in excreta.

C = concentration of radioactivity in red cells.

W = total worms.

D = total days.

$$L = \left(\frac{\text{Average loss of red cells}}{\text{worm}} \right) / \text{day}.$$

Then

$$L = \frac{R}{C W D}.$$

The fact that very little iron derived from red blood cell hemoglobin is normally excreted in feces would probably make it unnecessary to correct this formula by a determination of the base line fecal iron output after vermifugation.

References

1. CRUZ, W. O., HAHN, P. F., and BALE, W. F. *Amer. J. Physiol.*, 1942, **135**, 595.
2. HAHN, P. F. *Ind. eng. Chem., anal. Ed.*, 1945, **17**, 45.
3. HAHN, P. F. and BALE, W. F. *Amer. J. Physiol.*, 1942, **136**, 314.
4. HAHN, P. F., BALE, W. F., and BALFOUR, W. M. *Amer. J. Physiol.*, 1942, **135**, 600.
5. HAHN, P. F. *et al.* *J. exp. Med.*, 1943, **78**, 169.
6. HAHN, P. F. and WHIPPLE, G. H. *Amer. J. med. Sci.*, 1936, **191**, 24.
7. LEICHTENSTERN, O. *Dtsch. med. Wschr.*, 1886, **12**, 173.
8. WELLS, H. S. *J. Parasitol.*, 1931, **17**, 167.
9. WHIPPLE, G. H. *J. exp. Med.*, 1909, **11**, 331.

Prevention of Dietary Fatty Livers by Exposure to a Cold Environment¹

E. A. Sellers and Rosemary Wen You

Department of Physiology, University of Toronto, Toronto, Canada

In studies of the lesions which develop due to a deficiency of the lipotropic factors, it has been shown on numerous occasions that the development of fatty livers or of hemorrhagic kidneys is closely linked to caloric intake and to metabolic requirements. Severe lesions are more easily produced when growth is rapid and when food intake is high. Inanition may protect the liver and kidneys of an animal subsisting on a deficient diet. In an environment one or two degrees above freezing, the caloric requirement is increased greatly, as indicated by increased oxygen consumption and increased food intake. When rats weighing more than 150 g are exposed to such an environment they usually survive and some growth occurs, but at a slower rate than normal.

Two groups of ten male rats (Wistar strain), bred locally and weighing from 170 to 200 g, were given a diet ad libitum which permitted good growth but was deficient in choline and its precursors. One group was exposed to a temperature of $2.5 \pm 1^\circ \text{C}$ for a period of two weeks, while the other was maintained for the same period under similar conditions but at a temperature of

¹ These experiments form part of a project supported by a grant from the Defence Research Board of Canada. We are indebted to Prof. C. H. Best for his continued interest, and to Drs. Jessie Ridout and Jean Patterson for the estimations of liver lipids.

$25 \pm 2^\circ \text{C}$. At the end of the two-week period the animals were sacrificed, and their livers were examined chemically and histologically.

As might be expected, the fat content of the livers of the control group was high, averaging $24.8 \pm 4.90\%$ (total lipid expressed as % of wet weight). The average value found in the group maintained at 2.5°C was $7.2 \pm 1.24\%$. The average weight of the livers was approximately the same, but the weights of dry fat-free residues of the livers of rats kept in the cold environment were significantly higher than those of the control group. Although rats in the cold room ate more (average 22 g/day) than the controls kept at room temperature (average 15 g/day), their increase in body weight was less (average 1.3 g/day) than that of the control group (average 3.5 g/day).

The prevention of excessive deposition of fat in the liver in spite of increased consumption of a severely hypolipotropic diet would seem to be associated with the greatly increased total metabolic rate. The results of further study of this finding may throw light on the mechanism of action of choline, and perhaps on intermediary metabolic pathways which may be affected by exposure to a cold environment.

Films from Hemicellulose Acetates¹

Charles L. Smart and Roy L. Whistler

Department of Agricultural Chemistry, Purdue University, Lafayette, Indiana

Up to the present time, cellulose has been the only plant polysaccharide which has been acetylated for the production of commercial films and fibers. Commercially, hemicelluloses are separated from their natural mixture with cellulose and are regarded as undesirable impurities in pulp destined for esterification. Yet the major proportion of the hemicellulose mixture present in plants consists of xylan, a linear polysaccharide which should produce strong films. Consequently, an investigation was undertaken to obtain further information on the film-forming characteristics of hemicellulose acetates.

The hemicelluloses are sometimes isolated from crude plant material by extraction with alkaline solutions. However, lignin interferes, not only because it retards complete solution of the hemicelluloses, but also because some of it dissolves in the extract, causing difficulty in purifying hemicelluloses. These disadvantages are avoided largely through selective removal of lignin with a maximum retention of unchanged polysaccharides. Such delignified pulps are termed holocellulose (6).

The corneob is a typical example of hemicellulose-rich material. Approximately 80% of the corneob consists of polysaccharide material, one-half of which is cellulose, whereas the remainder is made up of a mixture of hemicelluloses. The entire polysaccharide mixture or holocellulose can be prepared by a modification (3) of the

¹ Contribution by Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Journal Paper No. 401.

usual sodium chlorite procedure (5). Practically all of the hemicelluloses may be removed from holocellulose by extraction with a 10% solution of NaOH. Analyses indicate that the residue is almost pure α -cellulose.

Neutralization of the alkaline hemicellulose solution causes precipitation of the higher molecular weight polysaccharides and leaves in solution most of the glycuronans and polysaccharides of comparatively low molecular weight. The precipitated material is called hemicellulose A. Most of the soluble material remaining in the neutral solution is precipitated by the addition of two volumes of alcohol and is called hemicellulose B (2).

Results in this laboratory show that practically all of hemicellulose A consists of xylan. The mixture contains only 3% of combined hexuronic acid anhydride. Hemicellulose A, 25% of the total holocellulose, can be esterified with acetic anhydride in the presence of 0.25% of nitric acid (4) to produce a white fibrous acetate containing 38.6% of acetyl groups (calculated for diacetyl xylan, 39.8%). The acetate is soluble in dioxane, pyridine, or in a chloroform-methanol 9:1 mixture. When the acetate is cast from any of these solvents, clear films are produced which show an average tensile strength of 7.2 kg/mm² when measured on a Scott IP-4 inclined-plane serigraph. Films from commercial cellulose triacetate, when prepared in a similar manner, have a tensile strength of 8.6 kg/mm². Films cast from solutions of mixtures of hemicellulose A acetate and cellulose triacetate are clear and strong provided that incorporation of hemicellulose A acetate does not exceed 50%. Larger amounts of hemicellulose A acetate produce cloudy films.

Hemicellulose B, 6% of which is glycuronan material, may be esterified easily by a mixture of acetic anhydride and pyridine, provided that it is first swelled in formamide (1). The resulting acetate is a light brown, powdery material having an acetyl content of 35.0%. When cast from a chloroform solution, it produces clear films having a tensile strength of 7.5 kg/mm². While films can be produced by mixing hemicellulose B acetate with either cellulose acetate or hemicellulose A acetate, they are all cloudy, indicating the immiscibility of hemicellulose B acetate with either of the other two acetates.

These results show that the presence of hemicellulose A acetates does not have an adverse effect on the production of clear films of high quality. It is only the hemicellulose B acetates and possibly other low molecular weight polysaccharides that are responsible for cloudy films. The hemicellulose B fraction is present in most plant tissues in small quantities only. It represents approximately 10% of corncob holocellulose. It may be easily and completely removed through short extraction with alkaline solutions of 1-2% concentration. The residue obtained from corncob holocellulose after such an extraction may be acetylated and cast into good films.

These observations suggest the desirability of revising pulping techniques in order to retain more of the higher molecular weight hemicellulose fraction and thereby permit the use of these hemicelluloses, which are now discarded in commercial practice.

References

1. CARSON, J. F. and MACLAY, W. D. *J. Amer. chem. Soc.*, 1946, **68**, 1015.
2. O'DWYER, M. H. *Biochem. J.*, 1926, **20**, 656.
3. RITTER, G. J. and KURTH, E. F. *Ind. eng. Chem.*, 1933, **25**, 1250.
4. WHISTLER, R. L., BACHRACH, J., and BOWMAN, D. R. *Arch. Biochem.*, 1948, **19**, 25.
5. WHISTLER, R. L., HEYNE, E., and BACHRACH, J. *J. Amer. chem. Soc.*, 1949, **71**, 1476.
6. WISE, L. E., MURPHY, M., and D'ADDIECO, A. A. *Pap. Trade J.*, 1946, **122**, 35.

A Possible Explanation of Symptom Formation in Tobacco with Frenching and Mineral Deficiencies

Robert A. Steinberg, John D. Bowling, and
James E. McMurtrey, Jr.

*Bureau of Plant Industry, Soils, and Agricultural
Engineering, Beltsville, Maryland*

Studies with Maryland Medium Broadleaf tobacco seedlings in aseptic culture (3, 4) have disclosed that slight excesses of amino acids in the medium led to the formation of characteristic growth abnormalities and chloroses. The symptoms of toxicity covered a wide range but were specific for each amino acid. Admixtures in some cases led to appearance of new abnormalities. Most effective was L(-)-hydroxyproline (3 ppm), the seedlings being killed at 5 ppm. A close approximation of the extreme symptoms of frenching was produced with the natural amino acid, L(+)-isoleucine (100 ppm); the unnatural isomer being relatively ineffective. These growth abnormalities included inhibition of stem and branch elongation, accelerated development of the leaves in the axillary buds, and reticular chlorosis of newly expanded leaves, together with greatly reduced leaf laminae ("strap leaves"). Increased leaf number was a prominent feature.

Similar responses to amino acids have now been obtained with tobacco plants growing in water-culture and soil. Frenching of oriental Xanthi tobacco was obtained with DL-isoleucine at 20 ppm in water-culture. Partially sterilized soil required very large quantities, however, in order to produce the symptoms of frenching in Connecticut Broadleaf and Xanthi tobacco.

Large scale analytical studies with field plants of Maryland Medium Broadleaf tobacco have afforded some confirmation of the interpretation that excessive accumulation of free amino acids in the plant may be a primary cause of symptom formation in certain abnormalities. Free amino acids in leaf laminae of mildly frenched plants increased to a maximum of 121% above normal. J. L. Stokes of Merck and Company estimated the corresponding increase for L(+)-isoleucine to be 50%. Free amino acids in leaf laminae of plants showing symptoms of mineral deficiencies increased with nitrogen deficiency by 32%; phosphorus, 48%; potassium, 587%; calcium, 120%; magnesium, 283%; and boron, 27%. These values are maxima. They confirm and extend the