zero temperatures or during a warming from the lower temperatures to above $-15°$; (4) that the possibility of obtaining the vitreous state depends primarily on the velocity of crystallization; pure water could not be vitrified on account of its excessively high crystallization velocity,[3] while gelatin gels are capable of vitrification in proportion to the slowness of their crystallization, that is, in proportion to their gelatin concentration.[4] The relation between the possibility of vitrification and the crystallization velocity is due to the fact that the vitrifying procedure consists essentially in cooling rapidly enough to bring the temperature of the material across the zone of crystallization temperatures (from $0°$ to $-15°$) before the ice crystals have time to form.

This method of vitrification could be applied to entire plant leaves.[2] But preliminary experiments on the vitality of entire leaves gave results which suggested that some cells or tissues were alive, while some others were dead. We then investigated the tissues singly, beginning with the epidermis. This tissue can be obtained in monocellular layers thin enough for the extremely rapid cooling required, it stays alive a long time after being separated from the plant and it lends itself to several vitality tests. The present report is concerned only with investigations on the vitality of the epidermis.

Pieces of onion epidermis previously stained with neutral red and differentiated with potassium hydroxide—a procedure which allows the living and the dead cells to be distinguished by their color[5]—were mounted in small metal clips in such a way that each side of the tissue presented an exposed area of about 20 mm.^{2} The preparations were then immersed in liquid air. When removed therefrom and brought back, in air, to room temperature, the cells were all dead, as Becquerel, who made similar experiments on the same material, has also observed.[6] Comparing these results with those reported above on the vitrification of gelatin, we thought that the epidermal cells might behave as the gels containing 90 per cent. water which, to be vitrified, must either be reduced to a thickness of a few micra or lose some of their high water content. We then tried to dehydrate them by plasmolysis.

Stained pieces of epidermis immersed for about 15 seconds in a 5 per cent. solution of sodium chloride, were mounted as indicated above and, after removal, with a blotter, of the excess plasmolysing solution, they were immersed in liquid air. On being withdrawn from it, they were brought back, in air, to room temperature; the cells were all dead.

[3] J. H. Walton and R. C. Judd, *Jour. Phys. Chem.*, 18: 722, 1914.
[4] E. H. Callow, *Proc. Roy. Soc. A.*, 108: 307, 1925.
[5] B. J. Luyet, SCIENCE, 85: 106, 1937.
[6] P. Becquerel, *Comptes rendus Ac. Sc.*, 204: 1267, 1937.

Assuming that death might have resulted from a congelation during the too slow warming of the material in the atmosphere after its exposure to liquid air and that warming in a liquid medium would be more rapid on account of the better heat conductivity afforded by the liquid contacts, we immersed the preparations in the plasmolysing solution after their treatment in liquid air. Several cells were now found intact and could be deplasmolysed or plasmolysed to a further extent, when put in a more concentrated solution of sodium chloride.

If, instead of a 5 per cent., we used a 10 or 15 per cent. plasmolysing solution, before and after immersion in liquid air, more of the cells were intact.

The fact that, to save the cells from disintegration, one must use the same means as for saving a gelatin gel from crystallization, that is, (1) dehydrate them, (2) cool them rapidly, (3) warm them rapidly; and the fact that the further the dehydration the more resistant are the cells to injury or the gels to crystallization, suggest that the same fundamental process takes place in the two cases, in other words, that disintegration of protoplasm subjected to extremely low temperatures is due to crystallization and that any method, such as vitrification, which prevents crystallization, prevents protoplasmic disorganization.

BASILE J. LUYET
GREGORY THOENNES

DEPARTMENT OF BIOLOGY,
ST. LOUIS UNIVERSITY

INSULIN AND THE OXIDATION OF ETHYL ALCOHOL BY EXCISED DIABETIC LIVER TISSUE

A RECENT study in this laboratory of the action of insulin in increasing the rate of disappearance of alcohol from the blood has indicated that the acceleration of glucose oxidation by insulin catalyzes the oxidation of alcohol.[1] It seemed to us that conclusive information regarding this relation might be gained by a study of excised tissue from an animal completely deprived of its insulin supply by pancreatectomy. So far only liver has been extensively studied, since this tissue is probably responsible for the major portion of alcohol oxidation.[2, 3]

Cats were employed for this study because pancreatectomy in this species results in severe diabetes. The pancreas was removed aseptically under anesthesia; and the animals were given adequate food and laboratory care until the diabetes became maximal.

[1] B. B. Clark and R. W. Morrissey, *Am. Jour. Physiol.*, (Proc.), 123: 37, 1938.
[2] E. Lundsgaard, *Compt. rend. Trov. Lab. Carlsberg*, 22: 333, 1938.
[3] L. F. Lelior and J. M. Muñoz, *Biochem. Jour.*, 32: 299, 1938.

The animals were then sacrificed and 500 mgm of the excised liver tissue were suspended in 6 cc of saline phosphate pH 7.4 contained in glass-stoppered 125 cc Erlenmeyer flasks. Both the experimental flasks and control flasks containing killed tissue had identical quantities of alcohol approximating 2.5 mgm. All flasks were filled with oxygen and incubated for 3 hours at 37° C. with continuous agitation. After $ZnSO_4$ precipitation, the entire contents of the flask were subjected to steam distillation, and the alcohol in the distillate determined by Harger's method.[4]

Under the experimental conditions outlined, 19 observations on 8 cats disclosed that an average of 23.5 per cent. of the alcohol present was oxidized by normal cat liver tissue in 3 hours; individual experiments ranged from 16 to 39 per cent.

In marked contrast, 17 observations on liver tissue from 7 diabetic cats depancreatized for 72 to 96 hours revealed an average oxidation of only 3.1 per cent. with a range from −0.5 to 6.2 per cent. Since the maximal limit of error for the measurement of the alcohol under the conditions employed is 4 per cent., it seems probable that only very small amounts of alcohol were oxidized. The completeness of the diabetes was indicated by respiratory quotients slightly below 0.70 for excised kidney tissue from these animals.

Liver tissue from one cat depancreatized, but maintained on adequate insulin for 90 hours after pancreatectomy, revealed an oxidation of 36 per cent. This further indicates that insulin is the specific factor concerned.

Additional evidence was obtained in two experiments on animals that had been depancreatized 72 hours previously. A lobe of liver was removed under anesthesia, and then immediately afterward, 20 units of insulin were given to one cat and 14 units to the other. Approximately five hours later, the animals were sacrificed and another portion of liver tissue removed. The ability of the liver tissue from the same animal, with and without insulin, to oxidize alcohol was then compared. With the diabetic tissue there was practically no oxidation (+0.3 and −0.56 per cent.), but after the administration of insulin 29.4 and 27.5 per cent. of the alcohol present was oxidized.

While the oxidation of alcohol by normal cat brain is relatively small, preliminary experiments have suggested that oxidation by diabetic brain tissue is still less. We are now studying the effect of experimental diabetes on alcohol oxidation by the intact animal.

These experiments indicate that insulin is necessary for the oxidation of ethyl alcohol by cat liver tissue. It is not yet evident whether insulin acts specifically on the alcohol oxidation system or indirectly through carbohydrate metabolism. In a complete report to be published later, data concerning the mechanism of this action will be discussed.

BYRON B. CLARK
R. W. MORRISSEY
J. F. FAZEKAS

DEPARTMENT OF PHYSIOLOGY
AND PHARMACOLOGY,
ALBANY MEDICAL COLLEGE,
ALBANY, N. Y.

[4] R. N. Harger, *Jour. Lab. and Clin. Med.*, 20: 746, 1935.

VITAMIN E AND AVIAN NEUROLYMPHOMATOSIS

BUTLER and Warren[1] have reported that vitamin E, supplied by injection or feeding of cold-pressed wheat germ oil, resulted in quick recovery from a paralysis which they indicated to be *neurolymphomatosis gallinarum*. They also claimed that adding wheat germ oil to the diet reduced the incidence of related diseases. Their statements are not supported by any definite figures, although their study "involved about 1,000 paralyzed birds."

Those who come in contact with the industry recognize the seriousness of avian neurolymphomatosis and the allied neoplastic conditions. Such methods of reducing mortality to the extent claimed by Butler and Warren would be well worth the expense of the added wheat germ oil.

The writer has investigated the curative effect of wheat germ oil in a study of 41 fowls showing clinical symptoms of fowl paralysis from the research flocks of this department. Ten of these birds were treated by intraperitoneal injection, 21 by feeding, and 10 birds served as non-treated controls. The source of vitamin E consisted of two lots of cold-pressed wheat germ oil, one of which had been lecithinized to reduce rancidity. These birds were, for the most part, White Leghorns of from 9 to 12 months of age. The procedure included recording (1) the symptoms and date of entry to the test and (2) gross lesions found during a complete routine autopsy. All hens were kept in flat-bottom battery cages with food and water easily accessible. They were not removed for autopsy until extremely emaciated or completely paralyzed for more than one day. The duration of the tests varied from 3 days, in extreme cases, to more than three months in less severe cases.

Ten fowls received wheat germ oil injected intraperitoneally, 1 cc per day, later reduced to 0.5 cc per day. Three of these received a total of 2 cc per bird, 3 got 3 cc each, 2 got 4 cc each, and the other two received 8 cc each of the wheat germ oil. Eight of these 10 birds showed at autopsy gross lesions of neurolymphomatosis involving the sciatic, brachial or

[1] W. J. Butler and D. M. Warren, *Jour. Amer. Vet. Med. Assoc.*, 92 (N. S. 45): 204–206, 1938.