

of storing fleshy plant material without loss from rotting, freezing, respiration, moulding, enzyme action, etc., and that it can be carried out at low pressures and temperatures, mainly by cheaper mechanical operations, as distinguished from ordinary methods of drying. This will result in a large saving of fuel costs and will also give a better dehydrated product because of the low temperature and rapid drying. In the manufacture of starch from sweet potatoes, these last considerations are important in preventing physical and chemical changes in the starch before it is extracted.

It is obvious that, due to the removal of soluble substances in the juice by this process, wide claims for the dehydration of vegetables for food use should not be made. There may of course be special instances where the loss of nutrients or flavor with the juice would not be undesirable. Concentrating the juice and recombining it with the dried material does not seem to be feasible because of the high fuel cost. It appears, at present, that the principal applications will be in those cases where the loss of juice is not vital, such as in the manufacture of starch from either sweet or Irish potatoes and other similar processes.

The simplicity and low cost of this method suggest that it may be carried out in small-scale plants located at the source of the raw product. The dehydrated material can then be shipped economically some distance for further manufacture. This seems an effectual means of conserving surplus crops, cull vegetables and other farm wastes. In this way the method should become of considerable economic importance.

This investigation has been conducted by the Carbohydrate Research Division, United States Bureau of Chemistry and Soils, and financed by a grant of funds from The Chemical Foundation, Inc., of New York.

E. F. HOPKINS

LAUREL, MISS.

#### THE GROWTH AND CHEMICAL ACTION OF ACETOBACTER SUBOXYDANS UPON I-INOSITOL<sup>1</sup>

PREVIOUS communications from this laboratory<sup>2, 3</sup> have dealt with the preparation of sorbose and of dihydroxyacetone by the action of *Acetobacter suboxydans* upon sorbitol and glycerol, respectively. It seemed of special interest to test the action of the organism upon cyclic polyhydric alcohols, and i-inosi-

tol was the first compound of this type chosen for study. This compound is of biological interest not only because of its wide occurrence, but because Miller and co-workers<sup>4, 5, 6</sup> have shown the compound to be identical with Bios I. It was thought that if this compound could be biologically oxidized to ketone compounds, which might exist in reversible oxidation-reduction systems, some light might be thrown upon its rôle as Bios I. Moreover, this type of reaction would permit the preparation of cyclic polyhydric ketones not now available. The organism has been shown, in our laboratories, to oxidize the i-inositol to a compound which present results indicate to be a di-keto-i-inositol. Details of preparation and identification of the compound will be published later.

Some observations on the culturing of the organism on i-inositol are of special interest and are noted at this time. In preliminary experiments, a medium containing 3 per cent. of i-inositol and 0.5 per cent. yeast extract (Difco) was inoculated with a culture of *Acetobacter suboxydans* which had been grown on a sorbitol medium. Growth was good and the Schaffer Hartmann<sup>7</sup> titration showed the formation of reducing material. However, it was found that the organism could not be carried on the inositol-yeast extract medium beyond the third transfer. The addition of as little as 0.1 per cent. of sorbitol to the above medium permitted indefinite subculture and high conversion of the inositol into the oxidation product.

The factor involved in the above phenomena is not sorbose but is some other fermentation product of the sorbitol. The material is present in the filtrate obtained by separation of the bacteria from the medium by filtration through a Berkefeld filter; it is stable when heated for 10 minutes at 100°, but shows some slight deterioration when stored for three weeks at 30°. The bacteria, washed free from the fermented sorbitol medium, were unable to oxidize the inositol unless the filtrate was added. Detailed studies are in progress to determine the nature of this factor and whether it will affect the oxidation of other materials by the organism.

JOHN W. DUNNING  
ELLIS I. FULMER  
JAMES F. GUYMON  
L. A. UNDERKOFER

#### THE EXCRETION OF PREGNANDIOL IN THE TOXEMIAS OF PREGNANCY

DETERMINATIONS have been carried out on the urine of pregnant women for sodium pregnandiol glucuronide, an excretion product of progesterone, according

<sup>4</sup> G. H. W. Lucas, *Jour. Phys. Chem.*, 28: 1180, 1924.

<sup>5</sup> Edna V. Eastcott, *Ibid.*, 32: 1094, 1928.

<sup>6</sup> W. L. Miller, Edna V. Eastcott and J. E. Maconachie, *Jour. Am. Chem. Soc.*, 55: 1502, 1933.

<sup>7</sup> P. A. Schaffer and A. F. Hartmann, *Jour. Biol. Chem.*, 45: 365, 1920.

<sup>1</sup> Contribution from the Department of Chemistry, Iowa State College. This work was supported in part by a grant from the Industrial Science Research funds for the fermentative utilization of agricultural products. The i-inositol was kindly furnished by Dr. Edward Bartow, of the University of Iowa.

<sup>2</sup> E. I. Fulmer, J. W. Dunning, J. F. Guyman and L. A. Underkoffer, *Jour. Am. Chem. Soc.*, 58: 1012, 1936.

<sup>3</sup> L. A. Underkoffer and E. I. Fulmer, *Ibid.*, 59: 301, 1937.

to the method described by Venning.<sup>1</sup> In cases of toxemia of pregnancy values below those for normal pregnancy have been obtained. In all cases of severe toxemias, clinically pre-eclamptic, none of the compound could be recovered.

In all the normal pregnant cases studied at various months of pregnancy, the values obtained for sodium pregnandiol glucuronidate were in keeping with those reported by Browne, Henry and Venning,<sup>2</sup> with three exceptions. In one of these three cases, no sodium pregnandiol glucuronidate was recovered in the fifth month, in another none of the compound was recovered in the ninth month, and in the third, a lower amount than normal was recovered. The urine of these three patients, however, when extracted for the unconjugated form of pregnandiol by a new method, was shown to contain the free form of the compound, pregnandiol. This free form of pregnandiol was recovered in amounts somewhat lower than the expected normal values calculated on the basis of the amounts excreted of the combined form of pregnandiol in other normal cases at corresponding periods of pregnancy.

In the course of these studies it was found that sodium pregnandiol glucuronidate in the urine is unstable and begins to hydrolyze at room temperature within a week and is almost completely hydrolyzed at the end of two weeks. The compound is rapidly hydrolyzed if the urine is allowed to stand at 37 degrees Centigrade for twenty-four hours. This form of hydrolysis in incubated urine results in very little loss of pregnandiol. Free pregnandiol has also been recovered from the fresh urine in those cases mentioned above, and in some cases of toxemia, by the same method, as is used to recover the free form after hydrolysis.

The details of this method as well as the values obtained for both the conjugated and the non-conjugated forms of pregnandiol, in the study of a series of cases of normal pregnancy and of the toxemias of pregnancy will be reported shortly.

PAUL G. WEIL

THE JOHNS HOPKINS HOSPITAL AND  
THE JOHNS HOPKINS UNIVERSITY

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### A METHOD FOR STUDYING AND INFLUENCING CORTICO-HYPOTHALAMIC RELATIONS

THE immediate effects of direct hypothalamic stimulation have been shown by many investigators to be rise in systemic blood-pressure, change in respiratory rate, dilatation of the pupils, elevation of hairs and movement of the nictitating membrane and, in addition, slower appearing metabolic effects. I have devised a method by which hypothalamic stimulation can be effected through the intact skull without entering and damaging the nervous tissue and hence it is also applicable to the human.

The hypothalamus of the cat lies about the posterior part of the hard palate at a site easily located by stripping the mucous tissues and periosteum and finding a midline bleeding point, the source of which is a vein lying in a bony canal, a remnant of the embryological craniopharyngeal duct. With experience this point can be located without reflection of the soft tissues. A probe is simply passed down to the bone in the midline at this point, making a circular opening of several millimeters in diameter in the mucosa. The outer table is tapped for a few turns and a small bakelite core screwed in. This bakelite core contains a silver electrode covered with cotton moistened with silver chloride solution. The electrode simply makes contact with the surface of the bone. Its end pene-

trates the top of the bakelite and an insulated lead-off wire is looped over it. An ordinary indifferent electrode is applied to the shaved neck. Relatively weak interrupted currents from an inductorium evoke typical hypothalamic responses, mentioned above, while stronger currents produce muscle movements in addition. Pain-producing stimuli in the neighborhood and elsewhere do not evoke these responses. It is suggested that the electrode may be left in place and stimuli applied to the conscious animal, repeated when desired, in order to study the slower acting effects of hypothalamic excitation within the metabolic field.

The electrode in the bone close to the hypothalamus has been used to lead off currents to amplifiers of an electroencephalograph made by Mr. Franklin Offner under the direction of Professor Ralph Gerard. The resulting waves have been compared to the cortical brain waves. In the electrohypothalamogram there are few alpha waves and definitely characteristic slow regular waves of low voltage. Preliminary studies show that stimulation of the hypothalamus produces changes in the cortical waves of an excitatory character, increasing the voltage of the alpha waves which become clearer and devoid of spikes and increasing the frequency of the alpha bursts. After stimulation fast beta waves increase in the cortical lead. Immediately after stimulation through the hypothalamic electrode the amplifier is switched back and the result of stimulation has been found to be an increase in frequency and voltage of the slow hypothalamic waves. Preliminary pharmacological studies indicate that intra-

<sup>1</sup> Eleanor Hill Venning, *J. Biol. Chem.*, 119: 473, 1937.

<sup>2</sup> J. S. L. Browne, J. S. Henry and E. H. Venning, *J. Clin. Investigation*.