edition, 1880, pages 123 to 125, or to Dr. August A. Thommen's part 3 of "Asthma and Hay Fever in Theory and Practice," Charles C. Thomas, Springfield, 1931, pages 503 to 505. Dr. Campbell's theory is thus merely a repetition of Dr. Wilson's theory published with such confidence in 1873 and dealt with so ably by the pioneer in hay fever pollen research, Dr. Blackley.

A casual inquiry into the present-day methods of the preparation of pollen antigen would disclose the fact that the irritating element is obtainable in equal amounts from perfectly fresh pollen and from that which has been denatured and kept in storage for many years. It is not at all necessary for the pollen granule to touch the mucous membrane to cause the characteristic reaction. Instillation of active extracts of pollen will accomplish the same result with the same rapidity. Moreover, skin tests will show equally strong positive reactions, whether they be made with perfectly fresh pollen, with pollen which has been subjected to desiccation and kept for many years, with pollen which has been washed with ether or with extracts of pollen made by any of a score of methods, all of which would prevent any possibility of development of pollen tubes. Further research along this line would not seem to offer any worth-while possibilities.

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SPECIAL ARTICLES

THE INFLUENCE OF IODOACETIC ACID ON THE RESPIRATORY METABOLISM OF MAMMALIAN TISSUES[1]

IN 1929, Lundsgaard[2] observed that iodoacetate (IAA) inhibited the glycolytic mechanism, yet permitted muscular contraction to continue, a fundamental observation which caused a revolution in the concept of the chemistry of the contraction process in muscle. This work led to a further exploration of the nature of the IAA effect, with results that have been of considerable significance for the study of carbohydrate metabolism and the lactic acid cycle in muscle as well as in other tissues.

Although Lundsgaard[3] later pointed out that, with proper concentrations of IAA, the respiration of frog muscle and of yeast cells could be maintained at normal levels for some time, little was known of the nature of the foodstuffs oxidized under these conditions until Meyerhof and Boyland's[4] paper appeared, reporting that the R.Q. of such muscles, with 70 per cent. of the original respiration, was about 0.71, indicating virtual cessation of carbohydrate metabolism. When lactate was added to this preparation, respiration was stimulated, and the R.Q. rose to 0.95, showing oxidation of the added lactate. Krebs's[5] studies on the oxygen uptake of brain, testis and sarcoma under the influence of IAA furnished more support to the view that, in addition to inhibiting lactic acid formation, IAA prevented the oxidation of glucose, although it did not interfere with the oxidation of lactate. Quastel and Wheatley[6] also concluded that exposure of brain tissue to IAA rendered it unable to utilize glucose. These results have had a considerable influence in shaping the theories of the pathway of glucose oxidation in the cell, as well as on the interpretation of the mechanism generally known as the Pasteur-Meyerhof reaction. They would suggest that glucose or preformed carbohydrate may have to go through a lactic acid stage before it can be oxidized. They may also be interpreted as favoring the unitary theory of oxidation and fermentation which postulates a common initial aerobic and anaerobic pathway of carbohydrate degradation. When this pathway is interrupted by IAA, and no endogenous lactate is available for oxidation and resynthesis, oxidation of carbohydrate ceases. Finally, an inhibiting effect of IAA on the phosphorylating mechanism has been demonstrated by Lohmann.[7]

However, Stannard[8] and Saslow[9] have recently reported that non-nutrient R.Q.'s of normal and caffeinized frog muscle lay between 0.90 and 1.00, even though anaerobic glycolysis had been stopped by treatment with IAA and iodoacetamide. We have extended the investigation of the nature of the oxidative processes in IAA poisoning to mammalian tissues, using skeletal, smooth and cardiac muscle, as well as brain, from normal cats and dogs. The brain cortex, freed as much as possible of white matter, was minced. The cardiac muscle was cut into thin slices. Smooth muscle was obtained from the small intestine by a method (unpublished) developed by Eugene Cohen in this laboratory. The skeletal muscle was prepared by careful dissection of bundles of muscle fibers sufficiently thin for microrespiration studies. Prior to study in the Warburg apparatus, the tissues were aerated in Ringer solution, to permit the loss by diffusion of any lactate which

[1] From the New York Hospital and the Departments of Medicine and Physiology of the Cornell University Medical College, New York City. Supported in part by a grant from the Committee on Research in Endocrinology of the National Research Council.

[2] E. Lundsgaard, *Biochem. Zeit.*, 217: 162, 1930.

[3] E. Lundsgaard, *Biochem. Zeit.*, 220: 8, 1930.

[4] O. Meyerhof and E. Boyland, *Biochem. Zeit.*, 237: 406, 1931.

[5] H. A. Krebs, *Biochem. Zeit.*, 234: 278, 1931.

[6] J. H. Quastel and A. H. M. Wheatley, *Biochem. Jour.*, 26: 725, 1932.

[7] K. Lohmann, *Biochem. Zeit.*, 236: 444, 1931.

[8] J. N. Stannard, *Amer. Jour. Physiol.*, 119: 408, 1937.

[9] G. Saslow, *Jour. Cell. Comp. Physiol.*, 10: 385, 1937.

might have accumulated during their preparation. Chemical estimations were made of the lactic acid content of the tissues and medium at the beginning and end of the experiments to determine whether this substance could have served as a substrate. The respiratory measurements were made in Ringer-phosphate solution of pH 7.4, to which glucose and IAA were added as desired. Glycolysis was measured manometrically and chemically in Ringer-bicarbonate solutions exposed to an atmosphere of 95 per cent. nitrogen-5 per cent. carbon dioxide. Concentrations of neutralized IAA were chosen which abolished all but minimal amounts of glycolysis, yet allowed a satisfactory maintenance of respiration. The results of typical experiments have been assembled in Table 1.

To judge from the R.Q.'s obtained with brain and all three types of mammalian muscle, oxidation of carbohydrate appears to take place as readily in the presence of sufficient IAA to inhibit glycolysis as in its absence. Furthermore, not only is preformed carbohydrate oxidized under these conditions, but added glucose is as effective in elevating the R.Q. in the presence of IAA as in the controls. Chemical analyses showed that preformed lactate was not responsible for these elevated quotients.

These results are in accord with those of Stannard and of Saslow on frog muscle. The disagreement between our results and those of Meyerhof and Boyland, of Krebs and of Quastel and Wheatley may be due to the greater concentrations which these other investigators employed. It may well be that toxic effects were produced by the higher concentrations quite apart from the specific influence of the poison on cell metabolism. Furthermore, Krebs and Quastel and Wheatley depended entirely on measurements of oxygen consumption to judge of inhibition of carbohydrate oxidation. The effect of lactate on respiration and the R.Q. of IAA poisoned tissue, pointed out by the early workers, can readily be confirmed, but much of the significance of this observation would be lost in view of the persistence of the capacity to burn glucose under the same conditions.

Our experiments lend no particular support either to the theory that carbohydrate must go through a lactic stage before it can be oxidized, or to the unitary theory of oxidation and fermentation. Carbohydrate oxidation, at least under these conditions, appears to be independent of the anaerobic mechanism. Nor does IAA specifically affect the phosphorylating process, if esterification is a necessary preliminary to glucose oxidation. The mechanism of the specific action of IAA on intermediary carbohydrate metabolism warrants further investigation in the light of these findings.

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TABLE 1

Tissue	Respiratory Quotient				Oxygen Consumption				Inhibition of Glycolysis	Concentration IAA
	Non-nutrient	N.N. IAA	Glucose 0.2 per cent.	Glucose IAA	Non-nutrient	N.N. IAA	Glucose 0.2 per cent.	Glucose IAA		
					cu mm/moist mg/hr.				per cent.	
Skeletal muscle (dog)	0.86	0.89			0.31	0.21			94*	1/10,000
	0.94		1.03	0.97	0.26		0.26	0.17	98*	1/10,000
Smooth muscle (cat)	0.85		0.95	0.95	0.30		0.34	0.26	90†	1/10,000
	0.95		1.05	1.01	0.25		0.24	0.23	94†	1/10,000
Heart (cat)	0.87		0.96	0.99	0.33		0.37	0.37	90*	1/100,000
Heart (dog)	0.83	0.81	0.91	0.90	0.60	0.58	0.61	0.58	99*	1/75,000
Brain gray matter (cat)	0.97	0.98	0.97	1.12	0.42	0.35	0.66	0.44	97*	1/100,000
	1.06	1.09	1.02	0.96	0.34	0.30	0.61	0.47	94*	1/100,000

* Lactic acid determined chemically. † Lactic acid estimated manometrically.

CRYSTALLINE FACTOR I

OF the five known components of the vitamin B complex, three of them—thiamine, riboflavin and nicotinic acid—have been available in crystalline form. The other two,[1] factor 1 and factor 2, have so far been known as extracts. Factor 1 has now been crystallized by a procedure to be described elsewhere. The crystals are colorless rods which aggregate mostly as rosettes and sometimes in fan shapes. The dry crystalline material has a very slight yellowish tinge which is probably due to a slight amount of impurity. Dermatitis of the peripheral parts of the body of rats on factor 1-deficiency diets[1] involving the feet, paws, ears and areas around the mouth[2] is promptly cured with a daily dose of 10 micrograms of crystalline factor 1; 5 micrograms daily will clear up the dermatitis somewhat more slowly. Rats on factor 1-free diets, in which growth had ceased, responded immediately with gains in weight on administration of crystalline fac-

[1] S. Lepkovsky, T. H. Jukes and M. E. Krause, *Jour. Biol. Chem.*, 115, 557, 1936.

[2] T. W. Birch, P. György and L. J. Harris, *Biochem. Jour.*, 19, 2830, 1935.