ence will read through the lines of exquisitely expressed thoughts, and among the tables and charts of tested data, and recall the warmth, the nobility of presence, the generosity of attention and interest for others, the gentleness of his searching wit, the pervading wisdom of the man whose authorship is warrant of his distinction.

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SPECIAL ARTICLES

STUDIES ON INHIBITION OF FERMENTATION BY YEAST MACERATION JUICE

FERMENTATION of glucose by yeast maceration juice is dependent on the quantity of inorganic phosphate present and the state of activity of the juice. This dependence was studied by the manometric method of Warburg. The time of induction, the maximum rate of fermentation and the quantity of CO_2 formed served to characterize the course of the fermentation.

Addition of phosphate prolongs the time of induction and decreases the maximum rate of fermentation, and is generally followed by a diminution of CO_2 formation. Addition of phosphate augments the quantity of CO_2 formed only if the quantity of phosphate present is not sufficient to satisfy the equation of Harden. The inhibition by phosphate is different from that by fluoride, which does not prolong the induction period, although it may increase the inhibitory effect of phosphate. The inhibition by phosphate is independent of the quantity of substrate but increases with decreasing concentration of maceration juice.

A "pre-fermentation" reduces the inhibitory effect of phosphate. The inhibition is also reduced if the phosphate is added during the course of fermentation. The inhibition is least if the addition takes place during the period of maximum rate of fermentation.

The addition of acetaldehyde reduces the phosphate inhibition. A certain amount of acetaldehyde has an optimum effect. On the other hand, if fermentation is inhibited by phosphate, accumulation of acetaldehyde during the fermentation is greatly diminished.

Pyocyanine and cytochrome C + cytochromoxidase give a similar reduction of phosphate inhibition. In these cases a consumption of O_2 takes place, which, however, is very small in comparison with the additional formation of CO_2. The O_2-consumption is not inhibited by phosphate; on the contrary it is somewhat increased in the presence of higher phosphate concentrations.

To localize the interference by phosphate, decarboxylation of pyruvic acid and the formation of phosphoglyceric acid were examined. These processes were not inhibited by high quantities of phosphate, but were rather promoted by them. Analyses for phosphoglyceric acid revealed that its formation during the fermentation is inhibited by phosphate in the same degree as is the fermentation. After addition of acetaldehyde, however, its formation sets in nearly immediately and is not depressed following addition of phosphate.

The phosphate inhibition evidently affects one or more of the slower links following the formation of phosphoglyceric acid, rendering them still slower. In this way it inhibits the formation of acetaldehyde necessary to oxidize glyceric aldehyde phosphoric acid. Both the fact that phosphate renders the oxidation of glyceric aldehyde phosphoric acid more complete and the greater deficiency of acetaldehyde in the presence of more phosphate seem to be responsible for the greater O_2 consumption in the presence of pyocyanine or the cytochrome system.

The different degrees of inhibition obtained in the various periods of fermentation or after a "pre-fermentation," can be explained by varying formation and accumulation of acetaldehyde and by the different speeds of the inhibitable links.

The inactivation of the maceration juice, which depends on age, temperature and dilution, decreases fermentation in the same manner as the addition of phosphate. The rate of the inactivation is greater, the lower the concentration of the juice. It is not based upon a monomolecular reaction. During the first stages of inactivation (which may be rather prolonged at low temperatures or scarcely demonstrable in the neighborhood of 40° C.) the activity remains constant. In later stages the inactivation is accompanied by turbidity formation. During the inactivation an augmentation of susceptibility to phosphate inhibition takes place, whereas the inhibition by fluoride or monoiodacetic acid is not similarly augmented.

In the presence of phosphate the inactivation and the formation of turbidity are retarded. It may be supposed that the original phosphate content of the juice acts as a stabilizer against inactivation.

The addition of acetaldehyde reduces the effects of the inactivation as well as does a short "pre-fermentation." This indicates that the enzyme is able to reactivate itself to a certain degree by the fermentation process.

A detailed exposition of this investigation will be published in *Arkiv för kemi,* Stockholm.

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ON THE PORPHYRIN NATURE OF THE FLUORESCENT "BLOOD CAKED" WHISKERS OF PANTOTHENIC ACID DEFICIENT RATS

WEANLING rats fed a diet deficient in pantothenic acid develop, in a period of four to six weeks, peculiar symptoms characterized by the accumulation of red material around the nose and on the whiskers. Because of the superficial resemblance of this red material to coagulated blood the condition has been described as "nose bleed"[1] or "blood caked" whiskers.[2] However, no thorough studies on the chemical nature of the deposited red material have come to our attention. We have investigated the nature of the red material from a chemical point of view and have endeavored to trace its origin in the pantothenic acid deficient rat.

The red deposits on the nose and whiskers of pantothenic acid deficient rats exhibited a bright red fluorescence in ultraviolet light. An ultraviolet lamp (G.E.B.H.$_4$) was used as a light source. Hemochromogen tests showed that only a minute quantity of the material is hemoglobin. A large proportion was found to be a coproporphyrin. The heavy red deposit around the mouth and on the whiskers of several pantothenic acid deficient rats was collected by washing with water slightly acidified with acetic acid. Zeile and Rau's[3] modification of H. Fischer's extraction method for porphyrins was followed. The completeness of the extraction was controlled with the aid of ultraviolet light. The red fluorescent material was completely driven into 0.54 per cent. HCl (coproporphyrin fraction) and showed after washing with chloroform the following spectrum in 25 per cent. HCl: I 551 mμ II 570 mμ III 594 mμ; the spectrum of its copper salt in pyridine was : I 565 mμ II 528 mμ. These spectra were compared with those of a sample of synthetic coproporphyrin I[4] and its copper salt respectively and proved to be identical under the experimental conditions. The spectroscopic measurements were carried out with the aid of a Zeiss pocket spectroscope, equipped with a wave-length scale.

Since it has been shown that the Harderian glands in rats exhibit a red fluorescence, and contain and secrete porphyrin[5,6] it was assumed that the fluorescent material might pass to the nose and whiskers of the rats by way of the naso-lacrimal duct. An attempt to test this hypothesis revealed a fluorescent naso-lacrimal duct in a pantothenic acid deficient animal. It therefore appeared likely that the source of the porphyrin deposit was the Harderian gland. The Harderian glands were removed from eleven weanling albino rats; to insure complete removal of fluorescent tissue the operation was performed under ultraviolet light. The animals were then placed in all-glass cages and fed a diet deficient in pantothenic acid. Seven unoperated controls subsisting on an identical diet all developed fluorescent porphyrin deposits on the nose within six weeks. The eleven operated animals developed the other known symptoms of pantothenic acid deficiency, but no fluorescent red-colored material appeared on the nose, whiskers or fur.

Three grams of the fresh Harderian glands (from 17 animals) were extracted for porphyrins. In agreement with Derrien and Turchini[5] the main fraction appeared to be protoporphyrin. However, a very small fraction could be extracted with 0.54 per cent. HCl, and this was identified spectroscopically as coproporphyrin. The spectrum in 25 per cent. HCl was : I 553 mμ II 595 mμ; the spectrum of the copper salt in pyridine was I 565 mμ II 530 mμ under the conditions of our experiments.

We therefore conclude that the red deposit around the nose and on the whiskers of pantothenic acid deficient rats is not blood but coproporphyrin and that this is derived from the Harderian gland.

After this paper was submitted for publication it was noted that Chick, Macrae and Worden[7] had attempted to characterize the reddish exudate which accumulates on rats deprived of vitamin B_2 factors. Our own independent and more exhaustive investigations confirm their observation that the material is not blood, and that it contains large amounts of porphyrin. They state that the material contains protoporphyrin. The data submitted above identify the porphyrin washed from the whiskers and fur of our rats as coproporphyrin.

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