pounds decrease or destroy the potency of all gonadotrophins thus far investigated.

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THE SYNTHESIS OF PHOSPHOPYRUVIC ACID ON OXIDATION OF LACTIC ACID

THE formation of pyruvic acid from phosphopyruvic acid, and its further conversion into lactic acid, are well-studied stages of the anaerobic decomposition of carbohydrates, whereas the reversal of these reactions has not yet been fully investigated.*

It is known that lactic acid on oxidation yields pyruvic acid, whose further course of aerobic decomposition is known. We have established that pyruvic acid produced by the oxidation of lactic acid can be phosphorylated, giving rise to phosphopyruvic acid. The synthesis of phosphopyruvic acid has been effected in minced muscle tissue by adding to it sodium lactate with a good supply of oxygen. Along with the synthesis of phosphopyruvic acid there is a decrease in the amount of inorganic phosphate. The presence of phosphopyruvic acid was recognized by its instantaneous splitting in the presence of mercury ions, and also from its splitting by iodine in alkaline medium, giving rise to iodoform and inorganic phosphoric acid. As an example we may report the results of one of our experiments.

The muscle of a cat was minced by means of scissors. Out of this homogeneous mass the following samples were taken:

Sample A: 20 g of tissue incubated for 90 min. at 40° C. with a good supply of oxygen in 50 cc of 2 per cent. $NaHCO_3$ prepared from m/10 sodium lactate + 10 cc H_2O + 40 mg KH_2PO_4 (0.45 mg per 1 g of tissue).

Sample B: Prepared as sample A + 0.1 g of NaF.

Sample C: 20 g of tissue incubated for 90 min. at 40° C. in 2 per cent. $NaHCO_3$.

Sample D: Control sample, without incubation. 20 g of tissue were placed in a 7 per cent. solution of trichloracetic acid.

After the incubation the proteins were precipitated with 20 cc of 25 per cent. trichloracetic acid. Filtration and analyses were carried out after 20 hours standing in the refrigerator.

Phosphopyruvic acid is absent in samples C and D. It must therefore have been absent from the tissue in its preformed state. Nor did it accumulate during the incubation without the addition of sodium lactate and without oxygen supply.

TABLE 1

IN MG OF H_3PO_4 – P PER 1 G OF MUSCLE TISSUE

Samples	H_3PO_4 – P after hydrolysis in nHCL at 100° C.					Decrease of the inorganic H_3PO_4 – P	Amount of H_3PO_4–P formed on hydrolysis in nNaOH within 30′ (triose-phosphate P′)	Amount of H_3PO_4–P formed on treatment of the protein-free-filtrate with 2nNaOH and I (phosphopyruvic acid – P + triose-phosphate – P)
	0′	7′	30′	60′	90′			
A	0.56	0.77	0.93	0.98	0.98	0.89	0.02	0.20
B	0.27	0.32	0.38	0.41	0.41	1.18	0.05	0.15
C	1.57	1.63	1.63	1.71	1.71	...	0	0
D	1.00	1.33	1.38	1.38	1.38	...	0	0

Sample A shows a considerable content of phosphopyruvic acid. The amount of acid actually formed in this sample is much larger than what may be inferred from the figure tabulated, as phosphopyruvic acid is in enzymatic equilibrium with phosphoglyceric acid (according to Meyerhof and Lohmann's[1] data, equilibrium in enzymic solutions at 20° C. is established at 29 per cent. phosphopyruvic acid and 71 per cent. phosphoglyceric acid). The decrease of the amount of the inorganic phosphate, and the accumulation of phosphate stable toward hydrolysis in nHCL, indicate that phosphoglyceric acid was formed along with phosphopyruvic acid.

The formation of phosphopyruvic acid in sample B is particularly conclusive. It is known that NaF blocks the reaction: Phosphoglyceric acid $\leftrightarrows$ phosphopyruvic acid. This rules out the possibility of phosphopyruvic acid being a product of the decomposition of glycogen under our experimental conditions.

In 1924 Embden and his co-workers[2] observed the decrease of inorganic phosphate on incubating a minced muscle tissue in the presence of lactate. They believed the anions of the lactic acid to possess a specific power for inducing the synthesis of hexosephosphate ("lactacidogen") in muscle. The incorrectness of this early opinion was proved by one of us (D.F.),[3] who found that a muscle tissue yields on incubation in lactate a compound which hydrolyses in nHCL in 30 min. and does not reduce $K_3Fe(CN)_6$. The data now obtained have elucidated the nature of the phosphoric compound thus produced by a muscle tissue on incubation in lactate.

There are reasons to think that lactate is not the only substrate for the synthesis of phosphopyruvic acid. Evidence of this is afforded by the investigations of Kalckar,[4] who observed the formation of phosphopyruvic acid in renal tissue on oxidation with malic acid.

* Editor's note: Compare, however, the papers of Meyerhof, Ohlmeyer, Gentner and Maier-Leibnitz (*Bioch. Z.*, 298: 396 (1938) and Green, Needham and Dewan, *Bioch. J.*, 31: 2327 (1937).

[1] Meyerhof and Lohmann, *Biochem. Z.*, 273: 60, 1935.
[2] Embden and co-workers, *Z. Physiol. Chem.*, 143, 1924.
[3] Ferdman, *Z. Physiol. Chem.*, 187: 160, 1930.
[4] Kalckar, *Biochem. Jour.*, 33: 631, 1939.

The significance of this process for the production of phosphopyruvic acid by the oxidation of lactic acid, and the mechanism of the phosphorylation, are being studied.

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CHEMOTHERAPY IN EXPERIMENTAL INFECTIONS CAUSED BY STREPTOBACILLUS MONILIFORMIS

STREPTOBACILLUS moniliformis is constantly associated with a pleuropneumonia-like organism[1] which may represent a phase in the life history[2] of the *Streptobacillus moniliformis*. In view of a report that gold compounds are effective against infections produced with certain pleuropneumonia-like organisms in rats and mice,[3] it was considered of interest to see whether or not these compounds would also affect experimental infections induced by *Streptobacillus moniliformis*. Such has proved to be the case.

The intra-abdominal injection of Swiss mice with moderate amounts of virulent young cultures of *Streptobacillus moniliformis* causes septicemia resulting in death, usually in from one to three days. The injection of minute amounts of virulent cultures or moderate amounts of old or attenuated cultures frequently causes arthritis involving one or more joints. The virulence of cultures is subject to fluctuations, so that all therapeutic experiments must be well controlled. The microbes used in these experiments were contained in young cultures grown on veal infusion broth containing 20 per cent. horse serum. Swiss mice weighing approximately 20 gm were used and were found to be more susceptible to the infection than a strain of white mice of mixed origin. The gold preparation used was gold sodium thiomalate (myochrysine), which was administered intramuscularly in one dose, usually at the time of the injection of the bacterial culture. A single dose of 0.25 gm of the drug per kilogram of weight of the mouse, administered at the time of injection of the microbic culture, or twelve hours before or after, was effective in protecting against from 100 to 1,000 times the least amount of culture necessary to cause death in four out of five mice within three days.

Individual experiments consisted in the intra-abdominal injection of a given amount of bacterial culture into ten mice and treating five of these mice with the aforementioned drug. The mice were observed for at least twelve days after injection.

In one series of experiments involving 120 mice, the dose of the organism was such that of the sixty untreated mice, fifty-six died and one was afflicted with arthritis. Of the sixty treated animals, only two died and four were afflicted with arthritis. The dosage of drug in this instance was 0.25 gm per kilogram of weight of the mouse. The amount of the gold preparation used appears to be rather large, but it was well tolerated by the mice.

In another set of experiments involving thirty mice, the amount of drug was reduced to 0.025 gm per kilogram of weight of the mouse. In this series thirteen of the fifteen untreated mice died, whereas of the fifteen treated mice, four died and six were afflicted with arthritis.

Four different strains of *Streptobacillus moniliformis* were used in these experiments. One of these strains was isolated from the blood of a patient who recently had rat-bite fever and the other three strains were isolated from rats. There was no noticeable difference in the therapeutic effectiveness of the gold compound on the infections experimentally produced by the use of these different strains.

The *in vitro* effect of the gold compound on the growth of the *Streptobacillus moniliformis* was found to be slight as compared with the *in vivo* activity of the compound. Twenty per cent. serum-veal infusion broth containing 0.125 per cent. of the gold compound caused a twenty-hour delay in the appearance of growth. When the broth contained 0.25 per cent., the final growth was decreased by one half. This drug caused some precipitation of the proteins of this medium, which may explain the effect on the growth of the microbe.

The organism was found to grow well in a medium consisting of soluble starch, proteose peptone and a number of salts. This medium contained much less protein than did the serum-veal broth and in this instance a concentration of the gold salt to 0.01 per cent. flocculated the medium and inhibited growth.

No therapeutic result was demonstrable with neoarsphenamine. In various experiments the drug was administered in amounts of 0.015 and of 0.03 gm per kilogram of weight of the mouse. It was administered by intravenous and by intra-abdominal injection, in some instances at the time of injection of the bacterial culture and in some instances six hours later.

Attempts to protect mice against experimental infection by the administration of sulfapyridine in the food were unsuccessful.[4, 5] Mice were placed on a diet of ground food mixed with 0.5 per cent. sulfapyridine for twelve, twenty-four and forty-eight hours before injection of the bacterial culture. In experi-

[1] Emmy Klieneberger, *Jour. Path. and Bact.*, 42: 587, 1936.
[2] Louis Dienes, *Jour. Infect. Dis.*, 65: 24, 1939.
[3] G. M. Findlay, R. D. Mackenzie, F. O. MacCallum and Emmy Klieneberger, *Lancet*, 2: 7, 1939.
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[5] J. Litchfield, White, H. and E. Marshall, *Jour. Pharmacol. and Exper. Therap.*, 66: 23, 1939.