adding 1 cc Merck's superoxol a deep red color immediately appears. After one hour stirring, the red solution is acidified with dil. HCl and extracted with petrol ether. The now deep green petrol ether contains pure *o*-nitrosophenol. The aqueous layer remains deep red. It contains coppersalt of *o*-nitrosophenol sulfinic acid (see exp. 4).

Exp. 4 (without benzene)

0.5 g cuprous hydroxide is suspended in 200 cc dist. water in which 0.5 g benzene sulfo-hydroxamic acid was dissolved (pH = 4.4). The pH is now adjusted to 2.9. After putting 1 cc Merck's superoxol in the well-agitated solution, the brownish liquid turns deep red with a violet tint. After one hour, the liquid is acidified with HCl and shaken with petrol ether. Nothing goes into the petrol ether and it remains colorless. With ether, however, the *o*-nitrosophenol sulfinic acid can be extracted. The yellowish green ether solution gives characteristic colors with many metals, just like the free *o*-nitrosophenol. Deep red copper, green ferrous, greyish brown cobalt, red nickel and red mercury salts are formed.

From the four experiments described, one can notice that the radical, nitrosyl, has been formed in three different ways—(1) oxidation of $NH_2OH \cdot HCl$ with cupric ions, forming cuprous ions; (2) reduction of HNO_2 with cuprous ions; (3) NOH released from benzenesulfo-hydroxamic acid by adding copper ions plus H_2O_2.

In experiment 3, the NOH is partly captured by the benzene present in the reaction mixture and, therefore, *o*-nitrosophenol copper besides red *o*-nitrosophenol sulfinic acid copper is formed. On acidifying, the *o*-nitrosophenol goes easily into petrol ether while the free *o*-nitrosophenol sulfinic acid is only soluble in ether or ethylacetate.

In experiment 4, the NO and OH substitute hydrogen atoms in the benzene ring of the formed sulfinic acid (the exact position of the chelate NO–OH grouping will be later determined).

Without going into detail here as to the mechanism of the reaction, I assume that primarily both NOH and benzene (toluene, ethylbenzene, xylene, etc.) are coordinately linked to the cuprous ion and thus activated by rearrangement of the the electronic system. The whole reaction occurs, so to say, in the inner sphere of the Werner copper complex, which might be a cuprous or a cuprous-cupric complex. However, only the cuprous central atom of the complex seems to be able to link benzene coordinately. On oxidation to the cupric form it is again released. The reaction between the activated benzene and nitrosyl could be written schematically in the following way:

Compound II is autoxidizable and forms Compound III. The cuprous-cupric complex is stable only in a small range of pH (2.1–4), and in this pH range the best results are obtained. Addition of small amounts of acetone or acetonylacetone to the reaction mixture prevents the formation of *o*-nitrosophenol from benzene entirely. By using the new reaction, we have already synthesized about sixty new *o*-nitrosophenol compounds, all of which show the characteristic of the chelate grouping, ortho NO–OH, towards metals. At the same time many interesting properties are developed by the different substitutes in the benzene ring in different (*o.m.p.*) positions of which we will have more to say in another paper.

OSKAR BAUDISCH
NEW YORK STATE RESEARCH INSTITUTE OF THE SARATOGA SPA, SARATOGA SPRINGS, N. Y.

THE FUNCTIONS OF DIPHOSPHOTHIAMINE (PHOSPHORYLATED VITAMIN B_1)

IN 1937, Lohmann and Schuster[1] identified *cocarboxylase* as diphosphothiamine. This important discovery, coupled with the work of Peters and his collaborators[2] on the necessity of vitamin B_1 for oxidation of pyruvate by the brain of avitaminotic pigeons, brought one more vitamin within the group of enzyme components. Soon after Lohmann's discovery, Lipmann[3] demonstrated that diphosphothiamine is one of the components of α-ketonoxidase, and Barron and Lyman[4] found that diphosphothiamine acted in animal tissues and bacteria as a catalyst not only for the decarboxylation and oxidation of pyruvate, but also for its dismutation.

The mechanism of this catalysis is still unexplained. It does not seem to be a reversible oxidation-reduction system, as riboflavine and nicotinic acid derivatives were shown to be, although such a theory was suggested by Lipmann.[5] In fact, a comparative study of the rates of reduction and reoxidation of thiamine and diphosphothiamine has shown that the vitamin becomes with phosphorylation more resistant to the action of reducing and oxidizing agents (Barron and Lyman[6]). Nor does it seem to act according to Langenbeck's theory,[7] for Stern and Melnick[8] have presented evidence against a "Langenbeck cycle" involving the amino groups in the pyrimidine ring. The multiple catalytic functions of diphosphothiamine suggest the possibility that it acts by forming the integral part of the activating protein of the enzyme systems concerned with the activation of pyruvate. Once the pyruvate is activated, it may react with catalysts for its oxidation, reduction, dismutation or condensation. This hypothesis need not postulate reversible oxida-

1 K. Lohmann and P. Schuster, *Biochem. Zeits.*, 294: 188, 1937.
2 R. A. Peters, *Chem. Weekblad*, 34: 26, 1937.
3 F. Lipmann, *Nature*, 140: 25, 1937.
4 E. S. G. Barron and C. M. Lyman, *Jour. Biol. Chem.*, 127: 143, 1939.
5 F. Lipmann, *Nature*, 138: 1,097, 1936.
6 E. S. G. Barron and C. M. Lyman. To be published.
7 W. Langenbeck, *Ergeb. Enzymforsch.*, 2: 314, 1933.
8 K. G. Stern and J. L. Melnick, *Jour. Biol. Chem.*, 131: 597, 1939.

tion-reduction; it predicts, on the other hand, more catalytic functions for the vitamin than those hitherto known.

To test the validity of this hypothesis, experiments were performed with white rats fed with a vitamin B_1 deficient diet. The degree of deficiency was followed by the loss of weight and by the determination of blood pyruvate. Rats fed with the same diet plus added vitamin B_1 were used as controls.

The mechanism of the synthesis of carbohydrates from pyruvate by liver slices, a synthesis discovered by Benoy and Elliott,[9] was first studied. If such a synthesis starts with the formation of phosphopyruvic acid from pyruvate and fumarate, aerobically:

$$\text{Pyruvate} + \text{fumarate} + H_3PO_4 + {}_3O_2 \rightarrow \text{Phosphopyruvate} + 4\ CO_2 + 2\ H_2O,$$

Phosphorylated vitamin may accelerate the synthesis of carbohydrate by activating the pyruvate in this primary reaction. Kidney slices of normal rats synthesized 9.93 mgs of carbohydrate per gram of fresh tissue in the presence of pyruvate (372 per cent. increase over the control) and 11.6 mgs in the presence of pyruvate plus fumarate (452 per cent. increase); the addition of vitamin B_1 did not increase these figures. Kidney slices of avitaminotic rats, on the other hand, synthesized only 5.35 mgs of carbohydrate with pyruvate (a 46 per cent. decrease compared to the control) and 5.15 mgs with pyruvate plus fumarate (55 per cent. decrease); on addition of vitamin B_1 there was a synthesis of 11.86 mgs of carbohydrate. In other words, vitamin B_1 restored to normal the rate of carbohydrate synthesis by the kidney slices of avitaminotic rats (Table I).

TABLE I

SYNTHESIS OF CARBOHYDRATE BY RAT KIDNEY SLICES*

Added substrate	Mgs glucose per gm fresh tissue	
	Normal	Avitaminotic
No substrate	2.10	2.54
Pyruvate (Pyr.)	9.93	5.35
Pyr. + fumarate	11.20	5.15
Pyr. + fum. + B_1	11.20	11.86

* Incubated for 3 hours at 38° in $NaHCO_3$-Ringer buffer with $O_2 : CO_2$ as gas phase; pH, 7.4. The values given are average values of several experiments. Pyruvate, 0.08 m.M.; fumarate, 0.04 mM; vitamin B_1, 50γ or 50 micrograms.

The next reaction studied was the formation of citric acid by pyruvate and oxaloacetic acid, a reaction discovered by Knoop and Martius:[10]

$$\text{Pyruvate} + \text{Oxaloacetate} + \tfrac{1}{2}\ O_2 \rightarrow \text{citrate} + CO_2.$$

Phosphorylated vitamin B_1 may accelerate the synthesis of citric acid by activating the pyruvate which takes part in this reaction. Chopped heart of control rats produced 3.32 mgs of citric acid after 30 minutes' incubation with pyruvate and malate (malate goes readily into oxaloacetate) and 3.39 mgs on addition of vitamin B_1. When fumarate was used instead of malate, there was 20 per cent. less citric acid formed. The synthesis of citric acid by the heart of avitaminotic rats was decreased by 50 per cent. with pyruvate and malate and by 73 per cent. with pyruvate and fumarate as substrates. The lack of increase in the synthesis of citric acid on addition of vitamin B_1 must be attributed to lack of its phosphorylation during the short time of its incubation. In fact, when rat kidney slices of avitaminotic rats were incubated with vitamin B_1 previous to the addition of substrates, there was an increase of 35 per cent. (Table II).

TABLE II

SYNTHESIS OF CITRIC ACID BY CHOPPED HEART AND KIDNEY SLICES OF RATS*

Added substrate	Mgs citric acid per gm fresh tissue	
	Normal	Avitaminotic
Heart—No substrate	None	None
Pyr. + malate	3.32	1.61
Pyr. + malate + B_1 ...	3.39	1.65
Pyr. + fumarate	2.64	0.75
Pyr. + fumarate + B_1 .	2.76	0.67
Kidney—No substrate		None
Pyr. + malate		0.60
Pyr. + malate + B_1 ...		0.81

* Incubated for 30 minutes at 38° in phosphate-Ringer; pH, 7.4; O_2 as gas phase; pyruvate, 0.134 m.M; l-malate and fumarate 0.186 mM. Total volumes 3 cc.

These *in vitro* experiments show that the synthesis of carbohydrates and of citric acid with pyruvate as one of the substrates is diminished in tissues from avitaminotic rats, and is increased on addition of vitamin B_1. They are offered as evidence for our view that vitamin B_1 is a catalyst not only for the oxidation and decarboxylation of pyruvate but also for many other reactions where pyruvate is one of the reacting substances.

E. S. GUZMAN BARRON
CARL M. LYMAN

UNIVERSITY OF CHICAGO

BIOELECTRIC POTENTIAL AS INDICATOR OF OVULATION IN THE HEN

IN order to discover bioelectric potential differences connected with the physiology of normal reproduction in the mature fowl a D. C. millivoltmeter[1] was applied to laying hens. The possibility of recovering the egg easily, as well as the fact that the fowl has only one functional ovary, made this species particularly suitable for the experiment.

While the experiments are still under way, some preliminary findings can be reported here.

[9] M. P. Benoy and K. A. C. Elliott, *Biochem. Jour.*, 31: 1,268, 1937.

[10] F. Knoop and C. Martius, *Zeits. physiol. chem.*, 242: 1, 1936.

[1] Modification by Dr. R. Parmenter of Burr, Nims, Lane apparatus.