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.

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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.

Using silver silver-chloride electrodes in saline solution as a medium of contact, two laying hens were tested in their potential difference between the two sides of the abdomen. Table I gives the results of the test.

TABLE I

BIOELECTRIC POTENTIAL DIFFERENCE IN 2 LAYING HENS AT DIFFERENT STAGES OF REPRODUCTION

Hen	Date	Hour	Last egg	MV	Next egg
S	June 6	1:15 P.M.	1:00 P.M.	− 3.8	
		1:25 "		−26.6	June 7 noon
		1:35 "		−19.0	
	June 7	2:00 "	12 noon	−11.8	
	" 8	10:10 A.M.		−19.8	
		11:00 "	10:40 A.M.	−18.4	
		11:10 "		− 9.2	
		11:20 "		+41.0	June 9 noon
		11:22 "		+ 9.2	
		2:26 P.M.		− 2.6	
	June 10	3:00 "		− 1.6	
	" 14	2:30 "		− 2.1	
L	June 6	1:10 P.M.	12:20 P.M.	− 6.3	
		1:20 "		− 6.3	
		1:30 "		−51.0	
	June 7	1:40 "		−10.5	
		1:55 "		−10.5	
	June 8	10:30 A.M.	8:00 A.M.	−10.5	
	" 10	2:50 P.M.	2:20 P.M.	−54.2	June 11 1:00 P.M.
		2:55 "		−50.4	
	June 14	3:00 "		+ 4.2	no egg
	" 17	2:30 "		− 4.2	" "

The non-ovulatory potential corresponded closely to the potentials of other species (pig, man, goat and dog) and there was a violent rise in potential difference at the time when ovulation must have occurred according to Warren and Scott (1934).[2] Tests were made frequently from the moment when an egg was laid to several hours afterward, and the peak in potential was found forty minutes in one case, twenty-five minutes in another case, and thirty minutes in the third case after oviposition. In both cases the ovum was recovered, that is, both hens laid eggs about 24 hours following their high potential. Two hours after ovulation it had reached normal, non-ovulatory levels.

While these results have to be repeated on a larger scale, it is hoped that some clue will be found as to the direction of the potential in connection with the one-sided ovarian function in the hen. So far, most readings have had negative values, but not all of them.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THERMOSTATIC CONTROL

THE few essential parts required are shown in the accompanying circuit diagram. It will be noticed that no batteries are needed, since the device is completely A.C. operated.

A dual triode tube of the 6A6 type (or equivalent) is employed with the plates and grids connected in parallel. In this circuit the tube acts as a half-wave grid-controlled rectifier. By the use of a bleeder resistor across the A.C. plate supply voltage the grid return through the thermoregulator circuit, TR, may be adjusted to such a value that the grid voltage becomes of sufficient magnitude in phase with the plate voltage to operate the relay, due to the rectified cathode current. The condenser stores sufficient energy and produces enough lag to keep the relay from chattering.

Any sensitive relay that will operate on 15 ma. or less and having from 2,000 to 5,000 ohms may be used. The circuit requires but one preliminary adjustment. Short the input (TR) leads and, starting from point A, move tap P along the bleeder resistance toward B until enough current flows through the relay coil to make it close. To insure reliability it is well to move P a little beyond this point. An alternative method is to merely insert a milliammeter in the plate lead at X and adjust P until the current reads that value specified for the type of relay used.

A "Sigma" type M relay of 2,000 ohms was used in one model of this device. It has been operating a

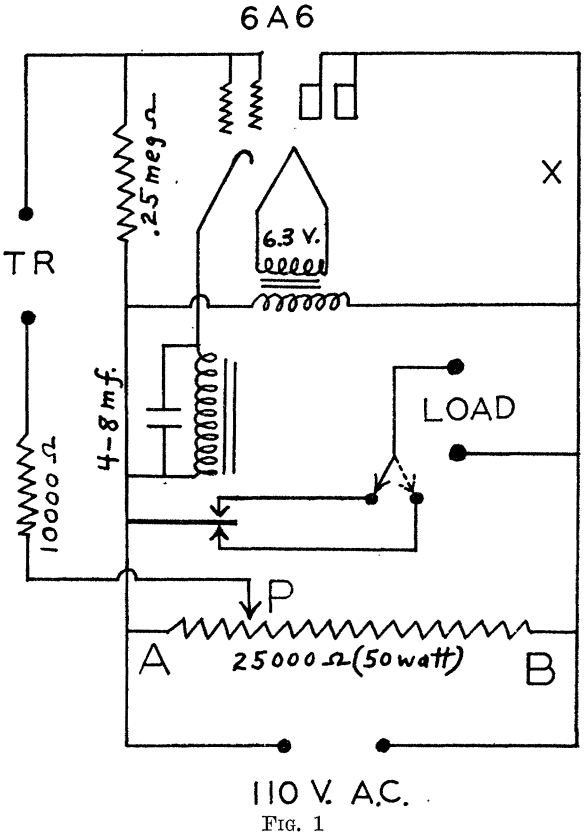

FIG. 1

[2] D. C. Warren and H. M. Scott, SCIENCE, 80: 461–462, 1934.