[6] O. NEUBAUER AND L. FLATOW, *Z. physiol. Chem. Hoppe-Seyler's*, 52 (1907) 375.
[7] E. L. ELIEL, D. E. RIVARD AND A. W. BURGSTAHLER, *J. Org. Chem.*, 18 (1953) 1679.
[8] M. F. S. EL HAWARY AND R. H. S. THOMPSON, *Biochem. J.*, 53 (1953) 340.
[9] M. D. ARMSTRONG, K. N. F. SHAW AND P. E. WALLS, *J. Biol. Chem.*, 218 (1956) 293.
[10] H. K. BERRY, H. E. SUTTON, L. CAIN AND J. S. BERRY, *Univ. Texas Publ.*, No. 5109, (1951) 22.
[11] J. F. GROVE, J. MACMILLAN, T. C. P. MULHOLLAND AND M. A. T. ROGERS, *J. Chem. Soc.*, (1952) 3949.
[12] E. W. BASSETT AND S. W. TANENBAUM, *Experientia*, in the press.
[13] E. L. TATUM, S. R. GROSS, G. EHRENSVÄRD AND L. GARNJOBST, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 271.
[14] S. GATENBECK, *Acta Chem. Scand.*, 11 (1957) 555.
[15] F. A. ISHERWOOD AND C. S. HANES, *Biochem. J.*, 55 (1953) 824.
[16] R. B. WOODWARD AND G. SINGH, *Experientia*, 6 (1950) 238.
[17] A. E. OXFORD AND H. RAISTRICK, *Biochem. J.*, 27 (1933) 634.
[18] E. D. AMSTUTZ, E. A. FEHNEL AND C. R. NEUMOYER, *J. Am. Chem. Soc.*, 68 (1946) 352.

Received October 17th, 1957

THE SIGNIFICANCE OF POTASSIUM AND SODIUM FOR THE SYNTHESIS OF GLYCOGEN IN THE ISOLATED RAT DIAPHRAGM

Z. DRAHOTA, M. KLICPERA AND R. ŽÁK

Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

The synthesis of glycogen at various K/Na ratios has been studied extensively by many authors, especially on slices of liver[1–5]. All the reports indicate that the optimal synthesis occurs in a medium containing a high concentration of potassium. A decrease of potassium ions in the medium leads in these experiments to a decrease in the synthesis of glycogen. TUERKISCHER AND WERTHEIMER[6] and STADIE AND ZAPP[7] have shown, however, that in the isolated diaphragm, optimal synthesis of glycogen takes place when there is a low concentration of potassium in the medium or when the medium is completely free from potassium.

In the experiments performed both on the liver slices and on the diaphragm, changes in the synthesis of glycogen were only determined in relation to the ion composition of the medium. The relationship between glycogen synthesis and the potassium or sodium content of the tissue was not studied. For this reason our attention was directed to the problem of the relation of glycogen synthesis to changes of potassium content in the tissue.

METHODS

Rats 80–100 g were used and the animals fasted for 24 hours prior to the experiment. The rats were decapitated and isolation of the diaphragm was performed according to RIESER[8]. In order to determine the glycogen synthesis in the same rat in a normal and modified medium, experiments were carried out with quarters of the diaphragm. It has been shown in a previous paper[9] that the diaphragm synthesizes glycogen to the same extent, irrespectively of whether halves or quarters were used. The ventral (thicker) quarter of the diaphragm was always used for the determination of the initial values of glycogen, while the dorsal (thinner) quarter was always used for incubation. It is necessary to adhere to this standard procedure, because the dorsal halves

References p. 35.

contain 16.5 ± 3.2 mg more glycogen per 100 g wet weight than the ventral halves. The diaphragm was weighed after isolation on a torsion balance and immediately transferred to an oxygenated warm medium (38°) and incubated in Warburg's apparatus. The interval between killing the rat and beginning the incubation was 5 min at the most. Cold preincubation, as recommended by some authors[10,15] was not used, nor was the tissue maintained at 0° during the preparation, since both the cold preincubation and preparation of the tissue at 0° cause changes in the ion composition of the tissue and its glycogen content[9].

Glycogen is expressed in mg of glycogen/100 g wet weight before the experiment. The synthesis of glycogen is given by the difference between the glycogen content before incubation (ventral quarter) and after incubation (dorsal quarter). Correction was not made for the difference in the glycogen content of the dorsal and ventral quarter. Glycogen was determined by a modification of the anthrone method[11]. The error of the method was ± 0.8 mg for values around 250 mg, *i.e.* 0.3 %.

As the experiments were carried out on rats 80–100 g in weight, left and right halves were used for the analysis of ions (*i.e.* 100–150 mg wet weight) instead of quarters. The potassium content of both halves of the diaphragm is the same. In one half the potassium and sodium content was determined prior to the incubation, in the other half following incubation. Sodium and potassium determinations were done with the Zeiss flame photometer. The diaphragm was dried to constant weight at 105° in a vacuum. Lipids were extracted from the crushed dry tissue by petroleum ether. Potassium and sodium were then extracted from the tissue by 1 % HNO_3 for 24 hours at 20°. The standards used for the potassium and sodium determinations were brought approximately to the contents of both ions in the tissue extracts, *viz.* sodium was added to potassium standards and potassium to sodium standards. All solutions used, were made in water redistilled in glass. Error of the determination was ± 2 % for 43.7 mequiv. K. and ± 3.5 % for 18.4 mequiv. Na.

Krebs-Ringer-bicarbonate and Tuerkisher-Wertheimer's solution were used as medium[6].

The concentration of glucose in both mediums was 200 mg in 100 ml. The tissue was incubated for two hours at 38° in 3 ml of the medium in Warburg's apparatus. The modified mediums were prepared by equivalent exchange of potassium by sodium and vice versa.

RESULTS

1. *Synthesis of glycogen in a medium with different concentrations of potassium*

During 2 hours of incubation the diaphragm synthesizes glycogen on both Krebs-Ringer-bicarbonate and Tuerkisher-Wertheimer's medium containing 200 mg% of glucose (see Table I). The average glycogen synthesis is higher in Tuerkisher-Wertheimer's medium, which is in accordance with data in the literature[6]. When potassium is equivalently replaced by sodium in the Krebs-Ringer-bicarbonate medium, the synthesis of glycogen in this modified medium is on the average 73% higher than in the normal Krebs-Ringer-bicarbonate medium. The increase of potassium concentration from 5.8 mequiv./l to 17.6 mequiv./l is without effect on glycogen synthesis. The synthesis of glycogen is therefore higher in a potassium-free medium. A similar conclusion was reached by TUERKISHER AND WERTHEIMER[6], whose experiments were confirmed by us. The glycogen synthesis is increased in a normal Tuerkisher-Wertheimer's medium, which contains 0.24 mequiv. of potassium per l. In a medium containing 17.6 mequiv./l, synthesis is lower. Our experiments have the advantage, as compared with the above experiments of TUERKISHER AND WERTHEIMER, that it is possible to determine the absolute increase of glycogen in a normal and modified medium.

2. *The changes in the potassium and sodium content of the diaphragm during incubation in mediums containing different concentrations of potassium*

During incubation in a normal Krebs-Ringer-bicarbonate solution containing 200 mg% of glucose, the diaphragm loses potassium (6.6 mequiv./100 g) and gains sodium (9.0 mequiv./100 g). The removal of potassium from the medium increases

References p. 35.

TABLE I

THE SYNTHESIS OF GLYCOGEN AT VARIOUS POTASSIUM CONCENTRATIONS OF THE MEDIUM

Medium	No. of samples	Normal medium				Modified medium				Synthesis modif./norm. medium
		Potassium in medium mequiv./l	Glycogen before incubation	Glycogen after incubation	Synthesis	Potassium in medium mequiv./l	Glycogen before incubation	Glycogen after incubation	Synthesis	
Krebs	4	5.8	271 ± 24	371 ± 26	100 ± 21	0.0	278 ± 9	451 ± 30	173 ± 26	1.73 ± 0.14
Krebs	3	5.8	176 ± 7	274 ± 9	107 ± 35	17.6	187 ± 19	295 ± 18	108 ± 8	1.01 ± 0.33
T-W	4	0.24	302 ± 23	540 ± 45	238 ± 26	17.6	290 ± 36	362 ± 35	72 ± 29	0.33 ± 0.10

Mediums: Krebs = Krebs-Ringer-bicarbonate, gas phase 95% O_2 + 5% CO_2; T-W = Tuerkisher-Wertheimer's medium, gas phase O_2. The modified medium was prepared by equivalent exchange of potassium for sodium or vice versa.

Glycogen: mg of glycogen/100 g wet weight. Synthesis: the increase of glycogen during incubation in mg/100 g. The diaphragm was divided into quarters. One half of the diaphragm (dorsal and ventral quarter) was utilized for the determination of the synthesis of glycogen in a normal medium and the other in the modified medium. The diference between the synthesis in normal and a modified medium is expressed as synthesis in the modified medium/synthesis in the normal medium.

TABLE II

CHANGES IN POTASSIUM AND SODIUM OF THE DIAPHRAGM AFTER INCUBATION IN MEDIUMS OF DIFFERENT CONCENTRATIONS OF POTASSIUM AND SODIUM

Medium	K in medium mequiv./l	No. of samples	K-mequiv./100 g FFW		Na-mequiv./100 g FFW		Changes during incubation	
			Before incubation	After incubation	Before incubation	After incubation	K mequiv./100 g	Na mequiv./100 g
Krebs	0.0	4	35.9 ± 1.31	24.5 ± 1.81	11.8 ± 0.35	28.4 ± 0.53	−11.6 ± 1.33	+16.5 ± 0.66
Krebs	5.8	11	42.3 ± 0.80	35.6 ± 0.84	15.4 ± 0.79	24.4 ± 1.05	− 6.7 ± 0.63	+ 9.0 ± 0.72
Krebs	17.6	4	45.5 ± 1.49	44.7 ± 1.64	15.9 ± 1.18	20.3 ± 1.51	− 0.8 ± 1.67	+ 4.4 ± 1.81
T-W	0.24	3	40.8 ± 0.48	21.7 ± 0.88	14.0 ± 0.32	26.9 ± 2.11	−19.0 ± 1.30	+12.9 ± 2.08
T-W	17.6	4	36.2 ± 0.63	29.0 ± 1.52	11.9 ± 0.53	25.5 ± 0.81	− 7.2 ± 1.07	+13.6 ± 0.29

Krebs = Krebs-Ringer-bicarbonate, gas phase 95% O_2 + 5% CO_2; T-W = Tuerkisher-Wertheimer's medium, gas phase O_2. Modified mediums were prepared by equivalent exchange of potassium for sodium and vice versa; the potassium and sodium content is expressed in mequiv./100 g fat-free dry weight (FFW); changes in potassium and sodium are given as the difference in the ion content in non-incubated and incubated hemidiaphragm.

these changes: loss of potassium from the tissue reaches 11.6 mequiv. K/100 g and gain in sodium is 16.5 mequiv. Na/100 g. The synthesis of glycogen under these conditions is concomitantly increased.

The increase in the potassium concentration of the Krebs-Ringer-bicarbonate solution to 17.6 mequiv. of potassium leads to a decreased uptake of sodium as compared with the normal medium. The potassium content remains unchanged. When the potassium content of the Tuerkisher-Wertheimer medium is increased, potassium loss is decreased and there is a similar decrease in the synthesis of glycogen. There are no changes to be noted in the sodium uptake (see Table II).

DISCUSSION

It was found that the synthesis of glycogen is influenced by the K/Na ratio in the muscle tissue. This effect of ions differs distinctly from that exerted on liver tissue. While HASTINGS *et al.*[4] and TENG[5] have stated that normal ion composition of the cells is necessary for normal metabolic activity (*i.e.* optimal glycogen synthesis) of the liver, our experiments indicate that greater synthesis of glycogen takes place in a medium, in which considerable changes in the ion composition take place during incubation. If the normal Krebs' medium, in which the diaphragm loses 6.6 mequiv. K/100 g of dry weight, is taken as the initial value, then an increase in glycogen synthesis takes place after decreasing the potassium content of the tissue. A similar relation holds in experiments with the Tuerkisher-Wertheimer medium (see Tables I and II), where following greater potassium losses from the tissue an increased synthesis of glycogen results. Changes in the sodium content of the diaphragm, when working with the Tuerkisher-Wertheimer medium, do not exhibit any relation to glycogen synthesis (Tables I and II).

A decrease in the potassium content and an increase in sodium take place during the first 15 min of incubation only, when using normal Krebs' medium. Later, the content of both ions remains constant[14,9]. During incubation in Tuerkisher-Wertheimer's medium or in potassium-free Krebs-Ringer-bicarbonate medium, the decrease in potassium, however, occurs throughout the course of the whole incubation. The synthesis of glycogen in both these mediums is higher than in the normal medium, where this continual loss of potassium is not present. The decrease in the potassium content of the diaphragm during incubation, therefore, leads to an increase in the glycogen synthesis.

It can also be concluded from our experiments that it is not possible to maintain with absolute certainty that during glycogen synthesis potassium enters the cell at the same time, and that the deposition of glycogen is linked to potassium accumulation, as VERZAR AND HASTINGS have attempted to show [12,13,14]. It can be especially well seen in the experiments with the Tuerkisher-Wertheimer medium in which the greatest synthesis is accompanied by the greatest potassium loss. This shows that glycogen can be synthesized without any increase in the total potassium content of the tissue.

SUMMARY

The synthesis of glycogen was studied at various concentrations of potassium in the diaphragm. It was found that glycogen synthesis is increased when potassium is lost from the tissue. During incubation in normal Krebs-Ringer-bicarbonate, 6.7 mequiv. K/100 g dry weight are lost and

References p. 35.

100 mg of glycogen/100 g wet weight are synthesized. Removal of potassium ions from the medium leads to a loss of 11.6 mequiv. K from the tissue, and 173 mg of glycogen are synthesized. An increase of potassium ions in the medium to 17.6 mequiv./l lowers the potassium loss from the tissue, but is without effect on the synthesis of glycogen. The role played by sodium in glycogen synthesis is discussed, together with questions connected with the synthesis of glycogen and the entrance of potassium into the cell.

REFERENCES

1 J. M. BUCHANAN, A. B. HASTINGS AND F. B. NESBETT, *J. Biol. Chem.*, 180 (1949) 435.
2 J. M. BUCHANAN, A. B. HASTINGS AND F. B. NESBETT, *J. Biol. Chem.*, 180 (1949) 447.
3 A. B. HASTING, CHING-TSENG TENG, F. B. NESBETT AND F. M. SINEX, *J. Biol. Chem.*, 194 (1952) 69.
4 A. B. HASTINGS, J. A. ASHRNORE AND G. F. COHIL, *Arch. Biochem. Biophys.*, 65 (1956) 78.
5 C. T. TENG, *J.-Lancet*, 73 (1953) 192.
6 V. TUERKISHER AND E. WERTHEIMER, *Biochem, J.*, 42 (1948) 603.
7 W. C. STADIE AND J. A. ZAPP, *J. Biol. Chem.*, 170 (1947) 55.
8 O. RIESER, *Biochim. Biophys. Acta*, 1 (1947) 208.
9 Z. DRAHOTA, M. KLICPERA AND R. ŽÁK, in the press.
10 D. H. BROWN, C. R. PARK, W. B. DAUGHDAY AND M. CORNBLATH, *J. Biol. Chem.*, 197 (1952) 167.
11 M. KLICPERA, Z. DRAHOTA AND R. ŽÁK, *Physiol. Bohemoslov.*, 6 (1957) 569.
12 E. LEUPIN AND F. VERZAR, *Helv. Physiol. et Pharmacol. Acta*, 8 (1950) C27.
13 R. PULVER AND F. VERZAR, *Helv. Chim. Acta*, 23 (1940) 1087.
14 E. CALKINS, I. M. TAYLOR AND A. B. HASTINGS, *Am. J. Physiol.*, 177 (1954) 211.
15 D. V. CLARKE, *Can. J. Biochem. Physiol.*, 33 (1955) 687.

Received September 27th, 1957

THE INHIBITION OF PHOTOSYNTHESIS BY SODIUM FLUORIDE

I. THE SODIUM FLUORIDE-INDUCED CARBON DIOXIDE BURST FROM *CHLORELLA**

NORMAN I. BISHOP AND HANS GAFFRON

Research Institutes (Fels Fund), University of Chicago, Chicago, Ill. (U.S.A.)

Selective inhibition of certain enzymic steps by fluoride–magnesium phosphate complexing would be a rather obvious means to look for possible differences between the well-known metabolic transformations of phosphoglyceric acid (PGA) in glycolytic systems and its photochemical reduction during photosynthesis. As we, and perhaps many others, have experienced, fluorides penetrate very slowly into green algae under the experimental conditions of approximately neutral media. The results indicated a rather complex disturbance of various parts of the general (cell) metabolism. Published experiments[1,2] have shown that photosynthesis is indeed sensitive towards the presence of fluorides in the cell. We found, for instance, after anaerobic fluoride treatment, an inhibition of fermentation and a decreased capacity for photoreduction. At times the inhibition was displaced by a period of photosynthesis in air; at other times such was not the case, *i.e.*, photosynthesis was completely inhibited[3].

* Work was supported by the U. S. Atomic Energy Commission, contract number AT(11-1)-239, and the Fels Fund. A summary of the results in this paper was presented before the Am. Soc. of Biol. Chemists, 1957. (See *Federation Proc.*, 16, No. 1, March 1957).

References p. 44.