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THE ENERGY OF UREA SYNTHESIS

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This study of the energy changes accompanying the synthesis of urea by living tissue from ammonia and carbon dioxide was undertaken in order to obtain more information regarding the causes of the specific dynamic action of protein; and because this reaction seems to be particularly suitable for the study of the energy changes in biological coupled reactions. In the synthesis of urea from ammonia and carbon dioxide (in which a large fraction of the ammonia is converted) there is a gain in free energy, which can be derived only from some other free energy liberating reaction.

Krebs and Henseleit[1] have shown that measurable quantities of urea are synthesized by liver slices prepared according to the method of Warburg.[2] Employing essentially their method we have confirmed this observation as well as their finding that a considerably greater rate of synthesis is obtained when ornithine is added to the fluid in which the slices are immersed.

Our immediate objective was a reëxamination of the relationship between oxygen consumption and urea synthesis. Krebs and Henseleit were unable to find, within the limits of their experimental error, any increase in oxygen consumption after ornithine was added to lactate Ringer's solution containing ammonia, even though the amount of urea synthesized was increased several fold. In their protocoll bearing on this question the rates of O_2 consumption (given as Q_{O_2}) were 20.5 and 23.9 in the experiment where the rate of urea synthesis (Q_{urea}) was 3.19, and were 21.1 and 20.4, where in the presence of ornithine, the Q_{urea} was 11.83. Because of these rather large variations in the rates of oxygen consumption we employed several modifications of their technique. These were the use of much larger amounts of liver tissue, quantities

varying from 20 to 50 mg. in each experimental vessel to reduce sampling errors; and further, each liver slice was cut into two approximately equal pieces, one being put into the control, the other into the experimental vessel. For the measurement of the oxygen consumption Krebs and Henseleit employed the Warburg two-vessel method.[3] In our experiments the CO_2 was absorbed by NaOH so that the O_2 consumption could be observed continuously, and the experiment terminated soon after the rate of respiration in either experimental or control vessel began to decline. The data given in table 1 show that though the concordance between duplicates was not as good in lactate as in glucose Ringer's solution, nevertheless in both types of solution the difference between duplicates was less than in the typical protocoll given by Krebs and Henseleit.

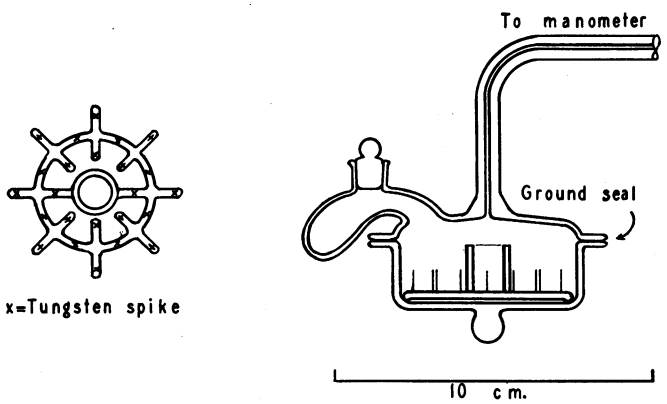

FIGURE 1

Diagram of manometer vessels and glass ring carrying the tungsten spikes on which tissue slices are mounted.

For these large quantities of tissue dessicator-shaped vessels were used, about 6.5 cm. in diameter, 3 cm. high and 74 cc. in volume. Their mode of attachment to the manometer and the seal are depicted in figure 1. The surfaces of the ground glass flanges constituting the seal were coated with lanolin-vaseline grease; and after the two parts of the vessel were put together, paraffin (m. p. 51–53°C.) was painted around the seal to make it gas tight. Elastic rubber bands passing under the lower part, and held by metal hooks on a removable metal ring on the upper part, took the strain off the paraffin, and helped to hold the seal tight.

The slices were carried on thin tungsten spikes fused into a glass ring which fitted snugly in the bottom of the vessel so that there was no sliding when the vessel was rocked. The glass ring was fused to a central well for the NaOH, the spikes and well being removable as one piece. We found this device more convenient, when many slices are to be used,

than glass spikes fused to the bottom of the vessel. Tungsten spikes, as far as we could detect, are not more injurious to liver slices than glass.

TABLE 1

RATES OF RESPIRATION OF SIMILAR LIVER SLICES IN TWO DIFFERENT VESSELS

TYPE OF RINGER'S SOLUTION	DURATION OF EXPT. MIN.	No. 1 WEIGHT OF TISSUE MG.	No. 1 Q_{O_2}	No. 2 WEIGHT OF TISSUE MG.	No. 2 Q_{O_2}
Glucose	50	31.4	8.67	30.4	9.05
"	20	24.4	8.66	25.8	8.86
"	60	36.6	8.74	36.6	8.83
"	25	36.2	8.80	35.0	9.20
"	15	57.2	8.91	55.0	9.36
"	40	61.4	10.57	64.2	11.15
Lactate	65	29.4	10.96	28.9	12.08
"	45	33.2	12.17	30.0	13.06
"	30	43.4	10.12	36.4	10.85

The composition of the Ringer's solution was that employed by Krebs and Henseleit; but ammonium bicarbonate was used instead of ammonium chloride as a source of ammonia. The gas was tank oxygen, and the

TABLE 2

UREA SYNTHESIS IN GLUCOSE RINGER'S SOLUTION

EXPT. NO.	DRY WEIGHT OF LIVER USED MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	DURATION OF UREA SYNTHESIS MINUTES	Q_{O_2}	Q_{urea}	ΔQ_{O_2}	ΔQ_{urea}	$\frac{\Delta Q_{O_2}}{\Delta Q_{urea}}$
1	31.4	0	0	90	7.52	0.26	1.49	1.55	1.0
	30.4	0	25		9.01	1.81			
2	24.4	0	0	105	9.36	1.13	0.31	0.51	0.6
	25.8	0	25		9.67	1.84			
3	50.2	0	0	120	8.06	0.79	1.89	2.08	0.9
	46.6	0	25		9.95	2.87			
4	36.6	0	0	80	8.40	0.38	0.66	0.72	0.9
	36.6	0	25		9.06	1.10			
5	36.2	3.3	0	50	7.39	1.05	1.63	2.04	0.8
	35.0	3.3	25		9.02	3.09			
6	32.6	0	0	85	12.48	0.38	0.53	0.49	1.1
	32.8	0	50		13.01	0.87			
7	33.0	0	0	95	6.64	0	0.82	1.26	0.7
	31.6	0	50		7.46	1.26			
8	50.2	6.5	0	165	9.37	0.40	3.81	4.98	0.8
	49.5	6.5	50		13.18	5.38			
9	37.9	6.5	0	145	7.66	0.23	3.82	4.70	0.8
	39.4	6.5	50		11.48	4.93			

experiments were carried out at 37.4°C. The urea was determined by the method described by Krebs and Henseleit, with the modification of

the addition of a small amount of freshly prepared haemoglobin solution to accelerate the CO_2 equilibration.

The animals used were white rats. The liver slices were prepared according to the manner described by Warburg. Their average thickness was about 0.3 mm. In a typical experiment the slices were mounted on spikes, placed in 15 cc. Ringer's solution, to which was added 1 cc. of ammonia, ammonia-ornithin or Ringer's solution. The stock ammonia, and neutralized *d*-ornithine solutions were made up in Ringer's solution.

TABLE 3

UREA SYNTHESIS IN *d-l* LACTATE RINGER'S SOLUTION

EXPT. NO.	DRY WEIGHT OF LIVER USED MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	DURATION OF UREA SYNTHESIS MINUTES	Q_{O_2}	Q_{urea}	ΔQ_{O_2}	ΔQ_{urea}	$\frac{\Delta Q_{O_2}}{\Delta Q_{urea}}$
10	29.4	0	0	105	9.80	0.30	1.69	1.17	1.4
	28.9	0	50		11.49	1.47			
11	33.2	6.5	0	80	10.82	0.94	2.64	4.29	0.6
	30.0	6.5	50		13.46	5.23			
12	34.2	0	0	110	12.52	0.33	3.49	1.82	1.9
	29.0	0	50		16.01	2.15			
13	19.6	0	0	100	13.68	0.16	1.65	1.46	1.1
	19.4	0	50		15.33	1.62			
14	34.6	6.5	0	95	9.09	0.39	2.92	3.32	0.9
	33.2	6.5	50		12.01	3.71			
15	27.2	6.5	0	140	11.23	0.25	2.59	3.44	0.8
	30.4	6.5	50		13.82	3.69			
16	18.0	6.5	0	90	9.96	0	2.94	3.38	0.9
	19.0	6.5	50		12.90	3.38			
17	28.0	6.5	0	90	10.79	0.20	4.76	4.40	1.1
	28.2	6.5	50		15.55	4.60			
20	31.0	0	47	100	12.45	2.34	2.91	3.97	0.7
	29.8	6.0	47		15.36	6.31			
22	19.2	0	50	130	14.27	1.37	2.64	2.75	1.0
	20.0	6.5	50		16.91	4.12			

0.5 cc. 10 per cent NaOH was added to the central well. For the urea determinations 10 cc. of solution were used. To this were added 1.5 cc. of acetate buffer and 0.3 cc. of freshly prepared 1 per cent human haemoglobin solution, with 1 cc. urease solution in the side arm.

With this technique we have found in every instance an increased oxygen consumption accompanying the synthesis of urea, in more than twenty-five experiments covering a variety of conditions (tables 2 and 3).

Each pair of figures shown in these tables was obtained from a different animal, there being only three vessels at our disposal for these experiments, of which one was necessarily the thermobarometer. The range of possible experiments was therefore restricted, and also the accuracy of the results.

Those obtained so far suggest that one molecule of oxygen is consumed for every molecule of urea which is formed, whether ornithine is added or not, and whether glucose or lactate Ringer's is used. For the present we are not inclined to attribute more than a qualitative significance to these results; and we shall postpone discussion of their bearing on the question of the energetics of the coupled reaction until after our experiments to obtain more concordant measurements, which are now in progress.

In spite of this qualification it seems justifiable to conclude even from these results that part of the specific dynamic action of protein must be ascribed to the superfluous energy released in the liver in the synthesis of urea from ammonia and carbon dioxide. The maximum contribution of this process is probably not more than 20 per cent of the total. This is in accord with the hypothesis proposed two years ago[4] that the increase in metabolism following the ingestion of protein is to be attributed to several processes, and that among these is the metabolism of the nitrogen.

TABLE 4

EFFECT OF ORNITHINE ALONE ON THE RATE OF RESPIRATION OF LIVER SLICES IN *d-l* LACTATE RINGER'S SOLUTION. ALL ANIMALS FROM SAME LITTER

EXPT. NO.	DRY WEIGHT OF LIVER TISSUE MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	PERIOD OF OBSERVATION MINUTES	Q_{O_2}	Q_{urea}
12	34.2	0	0	111	12.52	0.29
13	19.6	0	0	110	13.68	0.14
16	18.0	6.5	0	92	9.96	0
17	28.0	6.5	0	90	10.79	0.18

In the course of the experiments with ornithine we observed that in the presence of this amino acid the rate of respiration of the liver slices was sometimes much less than in the parallel vessel not containing ornithine. This did not occur always; and was more frequent and more marked in lactate than in glucose Ringer's solution. In most of the experiments the rats used were from eighteen months to two years old; toward the end only one litter three months old was available. The inhibiting effect of ornithine was most marked on the livers of these animals. Typical results are given in table 4. They show that if ornithine is not added to the control vessel, its inhibitory influence on the total respiration may completely obscure an additional oxygen consumption in the experimental vessel.

The rates of urea synthesis observed by us are in most cases less than those reported by Krebs and Henseleit. This probably was because the carbon dioxide tension in our experiments was much lower, nearly zero, as compared with 5 per cent in the experiments of Krebs and Henseleit. This point is also under investigation.

The quotient Q_{O_2} is cubic millimeters of O_2 (reduced to standard temperature and pressure) used per hour, divided by the weight of the tissue in milligrams after drying at 100°C. Q_{urea} is similarly the number of cubic millimeters of CO_2 (reduced to standard temperature and pressure) obtained after the action of urease, divided by the duration of the urea synthesis in hours, and the dry weight of the tissue in milligrams.

Summary.—(1) A modification of the Warburg technique is described facilitating the handling and measurement of the respiration of a large number of tissue slices in one vessel.

(2) With this technique employing rat liver slices, an increased oxygen consumption was found to accompany the synthesis of urea from ammonium bicarbonate in Ringer's solution.

(3) It is pointed out that this process is probably responsible for part (not more than 20 per cent) of the specific dynamic action of protein.

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ON THE INHERITANCE OF DIABETES MELLITUS

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Data have been collected on diabetes incidences in the families of 523 diabetic patients appearing at the Joslin Diabetic Unit from July, 1932, to October, 1932. A control group of 153 non-diabetic patients was similarly recorded. The data for diabetes incidences among parents and siblings of the patients in each group are given in table 1. Since diabetes incidence varies from decade to decade (see table 2, column 2), comparisons between the two groups are necessarily made by decades. It is obvious that the "diabetic" population contains a significant excess of diabetics among the parents and siblings. The statistical comparisons, using a 2×2 classification to test for independence for each decade, give a value of $\chi^2 = 18.366$, $P = 0.0095$ for the incidences among the parents and $\chi^2 = 22.153$, $P = 0.0047$ for the incidences among the siblings. Furthermore, 110 of the 523 patients or 22.94 per cent reported diabetic blood-relatives other than parents and siblings, whereas but 16 of the 153 non-diabetic patients, or 10.46 per cent, reported such diabetic relatives;