References and Notes

1. J. Genest, *Ciba Foundation Symposium* (Churchill, London, 1954), p. 203.
2. J. F. Tait, S. A. Simpson, N. M. Grundy, *Lancet* I, 122 (1952); S. A. Simpson *et al.*, *Helv. Chim. Acta* 37, 1163, 1200 (1954).

3. Work supported through grants of the Ministries of Health of Quebec and Ottawa (Federal-Provincial Plan), the Life Insurance Medical Research Fund, the National Research Council of Canada, and the Ciba Company, Montreal. We wish to express our gratitude for their collaboration and assistance to Fernande and Lucette Salvail, Pierrette Ledoux, Lise Langevin, Thérèse Ferguson, Aline Daoust, and Henry Schlegel; for calculations of the aldosterone equivalent to G. Russell, control laboratory, Ayerst, McKenna and Harrison Ltd., Montreal; for advice on statistical analysis and for many helpful suggestions to Vincent P. Dole, Rockefeller Institute, New York.

4. J. Genest *et al.*, *Proc. Montreal Physiol. Soc.* (1955); *Clin. Research Proc.* 3, 124 (1955).
5. I. E. Bush, *Biochem. J. London* 50, 370 (1952).
6. J. O. Irwin, *Suppl. J. Roy. Statist. Soc.* 4, 1 (1937).
7. J. Genest, *Can. Med. Assoc. J.* 73, 876 (1955).

* Fellow of the National Research Council 1954–55.

21 September 1955

Enhancement of Oxidative Phosphorylation of Glucose by Insulin

Polis *et al.* reported that under suitable conditions insulin enhanced oxidative phosphorylation of glucose by a preparation of liver mitochondria (*1*). This has been confirmed by us in experiments with rabbit tissue homogenates and extracts.

Rabbits were killed by stunning followed by decapitation, and the desired tissue (whole kidney or liver) was quickly removed and chilled on cracked ice for 2 to 3 minutes. The chilled tissue was homogenized in an all-glass (Pyrex) homogenizer (*2*) with 2 volumes of 0.067*M* phosphate buffer (Sorensen's) at *p*H 7.7 for 5 to 7 minutes at 0°C. The whole homogenate thus obtained was used in the majority of the experiments.

Table 1. Enhancement of oxidative phosphorylation of glucose by insulin in rabbit kidney homogenates and extracts.

Expt. No.	Inorganic P esterified (mg)	
	Without insulin	With insulin (4 units/2 ml)
Whole homogenates		
1	0.98	1.10
2	0.53	0.65
3	0.80	0.90
4	0.90	0.98
5	0.96	1.10
Dialyzed extracts		
6	0.82	0.91
7	0.51	0.59
8	0.42	0.56
9	0.50	0.55

In a few experiments, a dialyzed kidney extract was used. This was prepared as follows. Whole kidneys were homogenized as described in a previous sentence with 2 volumes of 0.1*M* phosphate buffer (Sorensen's) at *p*H 7.7. The homogenate was centrifuged at 2500 rev/min for 2 minutes and the turbid supernatant was dialyzed for 3 hours in a cellophane sack against 0.05*M* phosphate buffer at *p*H 7.7 and 1° to 2°C.

In experiments with whole homogenates, 1-ml portions of the homogenate were added to the main compartments of Warburg vessels that had previously been filled with 0.1 ml of 0.5*M* NaF, 0.2 ml of 0.5*M* sodium succinate and 0.5 ml of distilled water and standing on cracked ice. The side bulb contained 0.2 ml of 5-percent glucose. When the effect of insulin was tested, 0.1 ml of a 40-unit/ml plain insulin (Lilly) was added to the main compartment in the place of 0.1 ml of distilled water. The insulin was always added as the last addition. In experiments with dialyzed kidney extracts, 1-ml portions of which also were used in the experiments, the reaction mixtures contained, in addition to the afore-mentioned compounds, 0.1 ml of 0.5*M* $MgCl_2$ and 0.2 ml of 0.01*M* adenosine-5-phosphate.

Immediately after the additions had been made, the flasks were attached to the manometers and gassed with oxygen for 3 minutes at room temperature. They were then placed in the thermostat (38°C), and after temperature equilibration (7 minutes) the glucose in the side arm was tipped in and the flasks were shaken in the thermostat for another 20 minutes. The manometers were then taken out of the thermostat and the flasks were quickly immersed in ice water for a few minutes. The flasks were then detached, the protein was precipitated with trichloroacetic acid, and the material was filtered. The filtrates thus obtained were analyzed for their inorganic P content by the method of Fiske and SubbaRow (*3*) spectrophotometrically. The initial P contents of the reaction mixtures were determined in another set of flasks arranged and treated exactly in the same way as the experimental ones up to the stage of addition of glucose from the side arm.

Table 1 gives the results of the effects of insulin on oxidative phosphorylation of glucose by kidney homogenates and extracts. There was a small but definite increment in the amount of inorganic P esterified as a result of insulin treatment. Oxidative phosphorylation by the liver homogenates was also enhanced by insulin, and it was interesting to note that the enhancement was more pronounced with homogenates prepared from livers of alloxan-diabetic rabbits than with those prepared from the livers of normal rabbits (Table 2).

Table 2. Effect of insulin on oxidative phosphorylation of glucose by rabbit (normal and alloxan-diabetic, *4*) liver homogenates.

Expt. No.	Inorganic P esterified (mg)	
	Without insulin	With insulin (4 units/2 ml)
Normal rabbits		
1	0.94	1.25
2	0.76	0.98
3	1.00	1.18
4	0.97	1.26
5	0.58	0.78
6	0.69	0.81
Alloxan-diabetic rabbits		
7	0.35	0.50
8	0.64	1.04
9	0.73	1.25
10	0.64	1.14
11	0.63	1.05

In none of the experiments reported here did insulin have any effect on the oxygen consumption by the homogenates and extracts.

GANGAGOBINDA BHATTACHARYA*
Department of Physiology, University College of Science and Technology, Calcutta, India

References and Notes

1. B. D. Polis *et al.*, *Arch. Biochem.* 23, 505 (1949).
2. V. R. Potter and C. A. Elvehjem, *J. Biol. Chem.* 114, 495 (1936).
3. C. H. Fiske and Y. SubbaRow, *ibid.* 66, 375 (1925).

4. Rabbits were made diabetic by injecting 200 mg/kg of alloxan intravenously. Many rabbits could not stand the initial complications of alloxan injection and died within 3 to 7 days after the injection. These rabbits stopped taking food and became extremely emaciated. Such rabbits were not used in the experiments. Some of the rabbits, however, while showing a strong diabetes, as evidenced by high glycosuria and hyperglycemia, survived the initial complications due to alloxan injection. They consumed food in amounts comparable to those consumed by the normal animals and were not very much emaciated. Such rabbits only were used in the experiments. We did not resort to insulin treatment to help the rabbits overcome the initial complications and death due to alloxan injection.

* Senior research fellow, National Institute of Sciences of India.

12 September 1955

Finding of Silver Positive Reticulum in Early Human Tubercles

While the importance of silver positive reticulum in the structure of the tubercle is accepted, the site and time of its earliest appearance and what ultimately happens to the reticulum are not entirely clear. The literature on the subject, (*1–4*) points to its occurrence, in man, only in formed tubercles. Experimental