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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.

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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.

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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

evidence points to formation of reticulum fibers in tubercles between 1 week and 14 days following infection (*3, 5*). As far as can be determined from a careful survey of the literature, such early occurrence in human infection has not been reported. This is not surprising since most autopsied cases from which material is obtained are relatively late cases and have evidence of caseation necrosis.

Recently we were able to study (*6*) the spread of early tuberculosis in a 4-year-old child, who had died as a result of poisoning. In this case, the primary tubercle lay just beneath the mucosa of a small bronchus and induced a bronchial spread of the infection, with extremely minute and early tubercle formations ranging from a few lymphocytes and monocytes in the smaller alveolar ducts to early tubercle formations, quite like the early experimental infections produced by Opie (*7*). The photomicrographs of these new structures show well-formed reticulum being laid down as soon as a few monocytes and lymphocytes have gathered into a knot (Figs. 1 and 2).

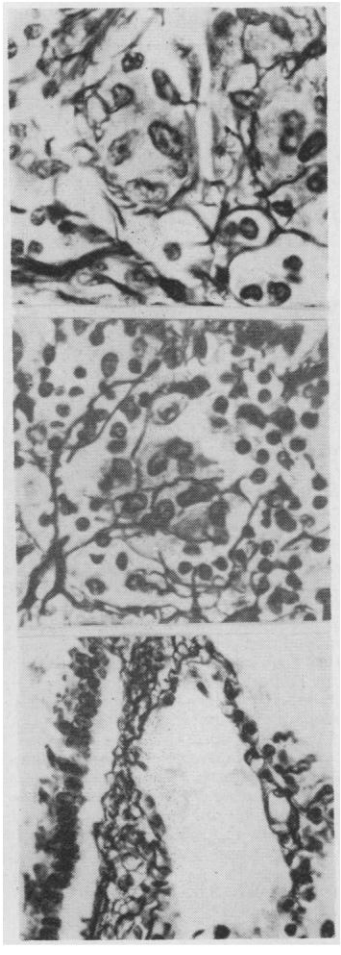

Fig. 1 (Top). An accumulation of large mononuclear cells and lymphocytes in an early tubercle. The reticulum is spread both over the surface of the mononuclear cells and between the cells. Note that some of the lymphocytes are meshed in the reticulum. Fig. 2 (Middle). Note abundance of reticulum surrounding and interlaced among the mononuclear cells. This is a pre-giant cell stage. Fig. 3 (Bottom). This section from an area of normal lung shows an arrangement of reticulum fibrils about a bronchiole continuous with fine fibrils in the alveolar walls. The reticulin stain used in these sections was that of Wilder (*9*).

Preparation of reticulum stains from normal lung areas in sites where the earliest tubercles are found show modest amounts of reticulum to be peribronchiolar and perivascular, and continuous in fine meshwork in alveolar walls (Fig. 3).

In the sections with tubercles, although reticulum is occasionally seen surrounding lymphocytes, our findings are similar to others in that the lymphocyte seems incapable of forming reticulum; however, once argyrophile fibers are laid down, lymphocytes may be found in their mesh. The reticulum fibers in our sections were around and among monocytes. Reticulum is found in the seven- to nine-cell accumulation stages of earliest identifiable tubercles and here appears at the earliest monocyte exudative phase. Observations of older tubercles are similar to others previously reported in that the fibers were most dense around the edge of the primary tubercle and where there are giant cells. In comparison of the reticulin stain with hematoxylin and eosin stains of the same lesion, we believe the reticulum fibers to be interjacent to but distinct from the fibrin. It was also interesting that under lower-power magnification, the distribution of the lesions in this case showed an intimate connection of the early tubercle lesions to small terminal bronchioles and alveolar duct walls. Acidfast stains on the lesions were positive for tubercle bacilli.

Miller (*1, 5*) reports the occurrence of reticulum in tubercles prior to formation of giant cells, but these tubercles are obviously in a more advanced stage than those reported here. Fresen (*2*), in 1950, in early human cases studied with silver stains, reported observations leading to the belief that epithelioid cells originated from reticuloendothelial cells and that epithelioid cells formed reticulum. Although reticulum formation has been extensively studied, most authors have not considered that reticulum occurs at the stage exhibited here.

Tuberculosis still remains an important infectious disease with a high mortality. Current work, such as that of Lurie (*8*) on the spreading effects of ACTH and cortisone, and also studies on chemotherapeutic agents in tuberculosis, should include investigation of the effects of these agents on early reticulum formation.

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Sweetpotato Internal Cork Virosis Indexed on Scarlett O'Hara Morning Glory

The internal cork disease of the sweetpotato (*Ipomoea batatas* L. Lam.) was discovered in the 1944 South Carolina crop and diagnosed as a virus disease by Nusbaum (*1*). Transmission of the necrotic spot root symptoms was demonstrated by root-plug grafting with an attending long incubation period of approximately 1 year. Since 1944 the disease has spread throughout most of the states growing the crop.

This situation posed the need for an indexing host with a shorter incubation period than sweetpotato. The prime essential was to find an indicator host better suited for studying the nature of the causal virus in order that more rapid progress could be made in studies designed for control of the disease.

By manipulation of the growth status and physiological behavior of peach seedlings, the incubation period of yellow-red virosis was shortened to 3 weeks from 1 to 3 years (*2*). Like the woody plant viruses (*3, 4*) for which grafting is often the only feasible means of transmission, internal cork virus has thus far been transmitted only by grafting. Ideally, an indexing host should be one

Fig. 1. Internal cork virus symptoms induced on Scarlett O'Hara morning glory by modified Yarwood technique (*7*): *a* leaf No. 4, 9 days after inoculation; *b* chlorotic condition that usually follows about 2 weeks later; *c* leaf No. 3 from base of stem and immediately below *a*, only leaf No. 1 having been inoculated.