the brains and livers of aggregated animals and that the total radioactivity found in brain extracts was quite similar in fasted (Table I) versus non-fasted (Table II) animals. We do not know at this time whether the increased ^{14}C found in the brains of aggregated mice or in those of isolated mice which received injections of LiCl is distributed uniformly among intermediary metabolites or whether it is incorporated specifically into certain pharmacologically-active substances (e.g., the amino acids glutamate and GABA).

Résumé. L'incorporation des atomes radioactifs de 1-^{14}C-pyruvate dans le cerveau des souris est plus marquée quand les animaux ont soumis à un isolement prolongé.

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Pancreatic Islet Cell Damage in Mice Produced by Coxsackie B_1 and Encephalomyocarditis Viruses[1]

It has been suspected that viral infection can cause diabetes mellitus in man[2–5], based on clinical evidence with isolation of viruses and determination of rising titers of neutralizing antibodies to certain viruses. Nevertheless, the pathogenesis of diabetes mellitus by viruses needs clarification.

In experimental studies, CRAIGHEAD et al.[6,7] reported diabetes mellitus or diabetes mellitus-like syndrome associated with islet cell damage of pancreas in mice infected with encephalomyocarditis (EMC) virus suggesting that the virus acts on the islets of Langerhans to reduce the mass of functional β-cells. But viral crystals have never been shown directly in the damaged β-cells of the islets of Langerhans. In our studies we located and demonstrated both EMC and Coxsackie B_1 virus crystals in β-cells of the islets of Langerhans in the pancreas of mice infected with these two viruses. The findings of significant islet cell necrosis with demonstration of viral crystals in the damaged β-cells of the islets of Langerhans of mice proved that viruses do produce islet damage.

The source of our EMC virus has been previously reported[8]. The Coxsackie virus B_1 was a virus stock received from the Communicable Disease Center in 1959. It has been passed once in KB cells and twice in monkey kidney cells.

Three groups of a random breed strain of HaM/ICR mice of different ages (new-born, 8-day-old and young adult) were used for EMC virus inoculation and 3 other groups of the same strain of mice (new-born, 14-day-old and young adult) were used for Coxsackie virus B_1 inoculation.

The mice were injected i.p. with 0.05 ml to 0.2 ml of EMC virus culture fluid with a titer of 10^{-6} $TCID_{50}$/ml or with 0.1 ml to 0.2 ml of Coxsackie virus B_1 culture fluid with a titer of 10^{-4} $TCID_{50}$/ml. Control mice were injected i.p. with the same amounts of virus-free culture fluids.

The EMC virus infected mice were killed 1 to 3 days and the Coxsackie virus B_1 infected mice were killed 1 to 8 days after viral inoculation. Control mice for each group were killed at the same times as the infected animals.

Histologically, all mice infected with either virus showed mild to severe pancreatic islet cell degeneration and necrosis, usually beginning on the first day after inoculation. The acinar cells also showed damage associated with interstitial inflammation of the pancreas. As time elapsed the lesions became more extensive and widespread. Generally, the acinar cell damage of the EMC virus infected mice was less severe than that of the Coxsackie virus B_1 infected mice. Atrophic changes of islets of Langerhans were noted in the Coxsackie virus B_1 infected mice 4 days after inoculation.

Electron microscopically, crystals of EMC and Coxsackie B_1 viruses were demonstrated in β-cells as well as in acinar cells of the pancreas along with significant ultrastructural changes. In most mice the damage to the pancreatic cells was so severe that it was virtually impossible to identify the cell type. In the identifiable β-cells of mice infected with EMC virus the damage was usually severe, whereas the damage to the β-cells of mice infected with Coxsackie B_1 virus was mild to moderate. With EMC virus infection, vacuoles and vesicles of unknown nature formed and the RER usually became dilated. There was a decrease in β-granules in some of the severely damaged β-cells. With Coxsachie virus B_1 infection, the damage was usually restricted to that portion of the cell in which the viral crystal was present. In the area surrounding the viral crystals, vesicles and vacuoles formed. The mitochondria appeared swollen and there was evidence that the β-cell granules had become dissolved and that the granule core was condensed. The pancreatic tissues of the control animals were normal.

That EMC virus infected mice have islet cell damage and develop diabetes mellitus-like syndrome has been shown[6,7]. Coxsackie virus B_1 infected mice showed islet cell damage similar to that of mice infected with EMC virus, but it is not known whether diabetes mellitus could also occur in Coxsackie virus B_1 infected mice. Biochemical studies of this nature are in progress in this laboratory.

The Coxsackie virus B_1 is one of the viruses known to infect man. Clinically, some diabetic patients have shown

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[2] E. GUNDERSEN, J. infect. Dis. *41*, 197 (1927).
[3] E. HINDEN, Lancet *1*, 1381 (1962).
[4] D. R. GAMBLE and K. W. TAYLOR, Br. med. J. *3*, 631 (1969).
[5] D. R. GAMBLE, M. L. KNESLEY, M. G. FITZGERALD, R. BOLTON and K. W. TAYLOR, Br. med. J. *3*, 627 (1969).
[6] J. E. CRAIGHEAD and M. F. MCLAND, Science *162*, 913 (1968).
[7] J. E. CRAIGHEAD and J. STEINKE, Am. J. Path. *63*, 119 (1971).
[8] G. E. BURCH, J. M. HARB, H. L. COLCOLOUGH and C. Y. TSUI, Arch. intern. Med. *127*, 148 (1971).

significantly high titers of neutralizing antibodies to Coxsackie B viruses[4,5]. It may be assumed, therefore, that Coxsackie virus B_1 can cause diabetes mellitus in man and other animals. The findings in the experiments described above provide strong evidence for a viral etiology of diabetes mellitus, since the direct viral invasion of β-cells of islets of Langerhans, followed by cell degeneration and necrosis and later atrophic changes, can finally reduce the mass of functional β-cells of the islets of Langerhans.

Zusammenfassung. In den β-Zellen der Langerhans'schen Inseln des Pankreas wurden Viruskristalle von mit Encephalomyocarditis-Virus oder Coxsackie-B_1-Virus infizierten Mäusen gefunden. Die Inselzellen zeigten sowohl leichte als auch schwere Schädigung.

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Effects of Benzo(a)pyrene on Isolated Rat Liver Mitochondria

In a previous study, the authors observed that hepatocytes of rats treated with benzo(a)pyrene underwent alterations of the endoplasmic reticulum and the mitochondria; these were, in particular, swollen, showed a pale matrix, rare cristae and an increase of the mannitol-impermeable spaces[1]. It was not clear, however, whether these mitochondrial modifications were due to the benzo(a)pyrene action directly exerted on the mitochondrial membranes or to a mediate or indirect action of the same toxic substance through the sites of other sub-structures of the hepatocytes. To clarify this histogenetic question the present communication reports the treatment of isolated rat liver mitochondria in vitro with benzo(a)pyrene.

The preparation of the mitochondria was carried out at 0 °C. The rats were killed and bled. The pools of livers previously minced and washed in sucrose (0.25 *M* and 0.44 *M*) were homogenized for 3 min in a Potter-Elvehjem homogenizer with a teflon pestle in the same sucrose solution. The homogenate was then centrifuged for 10 min at 5,500 *g*. The precipitate, consisting of the mitochondria, was resuspended in sucrose with the passages of the pestle carried out manually and then centrifuged for 10 min at 10,000 *g*. The mitochondrial pellet obtained was washed twice with sucrose 0.25 *M*.

The mitochondria thus isolated were resuspended and mixed in an electromagnetic agitator in an aqueous solution of 2% 3–4 benzo(a)pyrene for 30 min[2]. Immediately thereafter the mitochondria were washed with a sucrose solution 0.25 *M* and fixed in 3% glutaraldehyde diluted in 0.13 *M* phosphate buffer at pH 7.4 for 1 h, washed with buffer, post-fixed in phosphate buffered (pH 7.4) 1% osmium tetroxide with added sucrose for 1 h, dehydrated in ethanol and embedded in araldite.

From the material treated in this way, ultra-thin sections were prepared with a Porter-Blum MT2 ultramicrotome; these were then stained with uranil acetate and lead citrate and observed under an Elmiskop 1A electron microscope.

The mitochondria thus treated increased in volume and had a roundish shape, a pale and homogenous matrix,

[1] L. Cuccurullo and G. Manocchio, unpublished observations.
[2] A. Vescia, G.G. Giodano and G. Hermann, J. molec. Biol. *33*, 625 (1968).

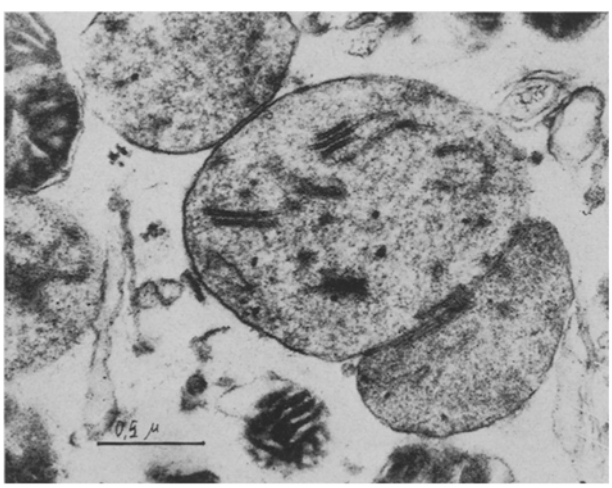

Fig. 1. Mitochondria treated increased, presented a pole, homogenous matrix and rare and broken cristae.

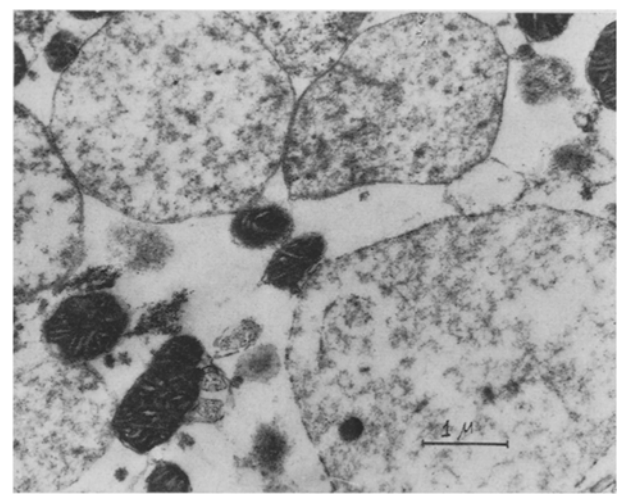

Fig. 2. The cytoplasmic matrix is swollen and in the mitochondria which reached striking volume it appeared rarefied.