Fig. 2. *Hep-2* cells: autoradiographs. Unstained ($\times 100$). A, control cells; B, 3 h after PR8 virus infection; C, 1 h after actinomycin action, typical 'rosette' around nucleoli; D, actinomycin-treated cells 3 h after infection

chicken fibroblasts and chorioallantoic membrane strips, ELD_{50} of PR8 virus as well as influenza viruses A/Asia/59, B/Lih/59 and B/95/61 was the same as in control cells. ELD_{50} of fowl plague virus was lowered in accordance with HA and CF antigen inhibition.

Thus actinomycin D seems to interfere with a cystron of a new synthesized viral RNA molecule which is responsible for viral proteins production; other explanations are not excluded.

A. G. BUKRINSKAYA

Institute of Virology,
Academy of Medical Sciences,
U.S.S.R., Moscow.

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VETERINARY SCIENCE

Levels of Coenzyme A in the Liver of Normal Cows and of Cows suffering from Primary Ketosis

HIGHLY significant changes have been shown in the levels of four members of the citric acid cycle (pyruvate, citrate, α -oxoglutarate and succinate) in the serum of cows suffering from primary ketosis¹. It was suggested that there may be interference with the reactions of the citric acid cycle, involving the conversion of pyruvate and α -oxoglutarate to citrate and succinate respectively. Furthermore, it was suggested as a working hypothesis that the cause of such an interference may be a deficiency of a co-factor or co-factors common to both oxidative decarboxylation reactions, such as coenzyme A. During the winter of 1962-63 coenzyme A levels were estimated in the livers of 7 cows suffering from spontaneous primary ketosis and compared with the corresponding levels in the livers of 7 normal cows at the same stage of lactation.

Cores of liver tissue weighing 500-800 mg were removed by the biopsy technique described by Loosemore and Allcroft². Immediately after removal, the liver core was

placed on filter paper to remove surface moisture, then dropped into a test tube standing in a bath of acetone and solid carbon dioxide. Protein was estimated on 100 mg of the frozen tissue by the method of Lowry, Roseborough, Farr and Randall³, while the rest of the liver core was transferred to a boiling tube containing 5 ml. water standing in a boiling water bath. The liver was boiled for 5 min, cooled, then homogenized in a Potter-Elvehjem homogenizer. The homogenate was made up to a known volume and centrifuged for 20 min at 20,000g in a Spinco model L ultracentrifuge. The coenzyme A content of the supernatant was estimated using a method based on the investigations of Stadtman, Novelli and Lipmann⁴, who showed that coenzyme A catalyses the interchange of acetyl bound and inorganic phosphate, and the arsenolysis of acetyl phosphate by a phosphotrans-acetylase enzyme system. The procedure adopted has been described in detail by Reece⁵.

It may be seen from Table 1 that there is a highly significant depression of the protein level of the ketosed liver. Therefore, although the level of coenzyme A (expressed as coenzyme A/g tissue) in the liver of the ketosed cow is lower than that found in the normal animal, no significant difference

exists between the two groups when the coenzyme A concentration is expressed in terms of the protein content of the normal and ketosed tissue.

Table 1. LEVELS OF COENZYME A AND PROTEIN IN THE LIVER OF NORMAL COWS AND OF COWS SUFFERING FROM PRIMARY KETOSIS

	Normal cases (7) mean $\pm$ S.E.M.	Pathological cases (7) mean $\pm$ S.E.M.	P*
Coenzyme A : LU/g tissue	94.6 $\pm$ 10.2	59.2 $\pm$ 14.8	0.1
Protein: mg/g tissue	185.0 $\pm$ 17.6	92.2 $\pm$ 8.5	0.001
Coenzyme A : LU/100 mg protein	51.5 $\pm$ 5.2	62.0 $\pm$ 11.5	0.5

* Probabilities calculated by the *t* test (ref. 10).

† Lipmann units.

No. of liver samples estimated given in brackets.

In view of these findings in liver tissue, the hypothesis that the interference in the oxidative decarboxylation of pyruvate and α -oxoglutarate is due primarily to a deficiency of coenzyme A appears to be unlikely. It has been suggested^{6,7} that this interference may be associated with other factors, such as deranged levels of the pyridine nucleotides, and other plausible arguments have been put forward⁸. However, it must be remembered that even in the normal cow there is a heavy drain on the coenzyme A reserves during the first 3 months of lactation due to the excretion of the co-factor in the milk⁹.

Until the levels of all the co-factors relevant to oxidative decarboxylation can be thoroughly investigated in the tissues of the ketosed animal, it will be difficult to explain the accumulation of pyruvate and α -oxoglutarate in the blood of cattle suffering from a starvation or a spontaneous primary ketosis.

K. G. HIBBITT

Institute for Research on Animal Diseases,
Compton, Newbury, Berks.

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