demonstrated. The results of Expt. 6 also indicate that the presence of 6-methylsalicylate in the reaction mixture repressed the endogenous formation of patulin from acetyl-SCoA precursors. This finding, and the fact that only trace amounts of 6-methylsalicylate itself were detected in Expts. 1 to 5 supports the contention that this aromatic compound is not an obligatory precursor of the antibiotic, but that it is in reversible equilibrium with a common acetyl-SCoA-derived open-chain intermediate.

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An enzymic study on the cellular origin of the DUNNING and the NOVIKOFF hepatomas in the rat

Because of technical difficulties that are difficult to surmount, the literature on the biochemistry of cancer is filled with comparisons of questionable validity. For example, in comparing liver cells and hepatoma cells, any such comparison can be questioned on the basis of whether the hepatoma cells have been derived from parenchymal liver cells*, from bile-duct epithelium, or from still other cells that occur in liver tissue. A number of studies summarized by POTTER[3,4] in relation to the "deletion hypothesis" have shown that several enzymes normally found in liver are either missing or present in very small quantities in the NOVIKOFF hepatoma, which has been regarded by some to be derived from parenchymal liver cells (discussed by NOVIKOFF[5]). Several of these enzymes were later reported by PITOT *et al.*[6] to be present in the DUNNING hepatoma and these authors questioned the significance of the earlier comparisons of NOVIKOFF hepatoma and liver, while further studies by DE VERDIER AND POTTER[7] with the DUNNING hepatoma revealed new differences between this tumor and normal and regenerating liver.

Abbreviations: 3'-Me-DAB, 3'-methyl-dimethylaminoazobenzene; dCMP, deoxycytidylic acid; Tris, tris(hydroxymethyl)aminomethane.

* That the parenchymal cells of the liver lobule differ quantitatively in their enzyme content depending on their anatomical location in the lobule has been shown by SHANK *et al.*[1] and by SCHUMACHER[2]. Such differences, which are small in comparison with the major fluctuations in enzymic activity shown by the liver as a whole, may reflect the blood flow and oxygen tension in various zones of the liver lobule[2].

An interesting example giving the appearance of the converse of deletion, *i.e.*, an enzyme not present in liver[8] but present in the NOVIKOFF hepatoma[9], was reported by MALEY AND MALEY, who found dCMP deaminase in the tumor but not in adult rat liver*. Earlier studies on regenerating rat liver by HECHT AND POTTER[10] indicated the use of a pathway from uridylic to thymidylic acid without the necessity for first forming deoxycytidylic acid, and made it unnecessary to assume that deoxycytidylic acid deaminase synthesis was suppressed in normal liver and released or induced in NOVIKOFF hepatoma and regenerating liver.

Prior to the present study DUNNING hepatomas were assayed[11] for dCMP deaminase and appeared to contain none of the enzyme. MALEY AND MALEY[9] reported slight activity of the enzyme in 24–48 h regenerating liver; however, we were not able to reproduce this result consistently. Rats bearing the DUNNING hepatoma were sent to MALEY and MALEY, who confirmed the absence of the deaminase from this tumor[12]. This finding left unexplained the occurrence of considerable amounts of the enzyme in the NOVIKOFF hepatoma[9], a result which we have confirmed. In order to test the hypothesis that the NOVIKOFF hepatoma is derived from a cell type other than that from which the DUNNING hepatoma stems, which could now be argued to be derived from a parenchymal liver cell on the basis of enzymes both present[6] and absent[11], we examined the livers from animals fed a diet[13] containing 0.06 % 3'-Me-DAB** for short periods of time sufficient to produce extensive bile-duct proliferation but no tumors, as shown by PRICE *et al.*[14]. As shown in Table I, these livers contained readily demonstrable amounts of the dCMP deaminase. When the animals were removed from the diet and fed laboratory chow for 2 weeks, the deaminase activity was not

TABLE I

All assays were performed on the S_3 fraction (microsome-free supernatant) prepared from 20 % isotonic KCl homogenates of the respective tissues. The incubation vessels contained 0.5 ml S_3 fraction, 0.2 ml 0.03 *M* dCMP (Calif. Corp. for Biochemical Research) dissolved in 0.2 *M* Tris–HCl buffer, pH 8.0, 0.1 ml 0.1 *M* KF, and 0.2 ml 0.154 *M* KCl[9]. The reaction time was 20 min. The reaction was stopped with 4.0 ml of 1 *N* $HClO_4$. After centrifugation to remove the precipitated protein, the supernatant was neutralized with KOH using phenol red as an indicator. The tubes were left at 0° overnight and the resulting crystals of $KClO_4$ were centrifuged down. The supernatant was decanted onto 6 × 1 cm columns of Dowex 1 in the formate form and the nucleotides and nucleosides separated and analyzed by the method of BRUMM AND POTTER[11]. The enzyme activities are expressed as μmoles deoxyuridylic acid formed/h/g tissue.

Tissue	*dCMP deaminase activity*
Normal liver	0, 0, 0
Embryonic liver (17–20 days)	58, 20
Regenerating liver (48 h)	0, 1, 5
DUNNING L-C18 hepatoma§	0, 0, 0, 0
NOVIKOFF hepatoma§§	70, 59, 66, 41
Liver, 3'-Me-DAB diet – 22 Days	11, 11, 11, 8
Liver, 3'-Me-DAB diet – 36 Days	7, 5, 8, 5
Liver, 3'-Me-DAB diet – 27 days, followed by 15 days Laboratory Chow Diet	0, 0, 0, 0.4

§ Maintained in Fischer strain inbred rats originally supplied by Dr. W. F. DUNNING, U. of Miami, Coral Gables 46, Florida.

§§ Obtained in 1956 from Dr. A. B. NOVIKOFF, Albert Einstein School of Medicine, New York City.

* We are indebted to MALEY AND MALEY for sending us their manuscript[9] prior to publication.

** These animals were kindly supplied by Drs. J. A. MILLER and E. C. MILLER.

measurable. Histologic sections taken at this time showed an almost complete disappearance of bile-duct proliferation*.

These results suggest the possibility that the NOVIKOFF hepatoma cell is derived from a cell that resembles bile-duct epithelium more than it resembles parenchymal liver cells, while the DUNNING hepatoma cell may be more closely related to parenchymal liver cells. It is of interest that despite their marked differences with respect to the deaminase, the two hepatoma strains resemble each other in their weak activity with respect to uracil and thymine deoxyribose transferring enzyme[7] and their inability to catabolize uracil and thymine. The latter results will be reported elsewhere. The biochemical indications for a relationship between the two hepatomas and their possible normal prototypes support a combined biochemical and pathological study undertaken by PITOT, CLARK AND FARBER [6](*cf.* PITOT[15]). DAOUST[16] has carried out biochemical and cytological studies on heterogeneous cell populations and perhaps by these or by additional techniques[1] further evidence for the derivation of these hepatomas, as well as others now under study, can be accumulated.

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