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EFFECT OF 5-AZACYTIDINE ON DIETARY AND HORMONE INDUCTION OF SERINE DEHYDRATASE AND TYROSINE AMINOTRANSFERASE IN RAT LIVER

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Abstract—Dietary and hormone induction of serine dehydratase and tyrosine aminotransferase in the liver of rats treated with 5-azacytidine has been studied by a specific immunoprecipitation technique. While the synthesis of serine dehydratase induced by cortisone or dietary tryptophan is depressed by 5-azacytidine administered simultaneously or after the inducer, the increase of tyrosine aminotransferase is not affected by the analogue administered after the inducer. 5-Azacytidine does not block the increase of tyrosine aminotransferase once it has started; moreover, the drug alone causes a similar enhancement of the enzyme activity. However, induction of tyrosine aminotransferase is partially depressed by the simultaneous administration of the drug with the inducer. The significance of the findings in view of the known inhibitory effect of 5-azacytidine is discussed.

5-AZACYTIDINE shows marked biological effects consisting in its ability to interfere with the synthesis of various types of nucleic acids.[1–5] This action is preceded by its phosphorylation to 5-azacytidine 5′-phosphate which affects orotidine 5′-phosphate decarboxylase[6,7] and is readily incorporated into various RNA fractions. It is assumed that the interference of 5-azacytidine with RNA synthesis results also in the changed protein and DNA synthesis which have been observed in various mammalian systems after the administration of the drug.[3,4,8–11]

While studying the action of 5-azacytidine on the synthesis of liver proteins, complete inhibition of hormone induction of tryptophan oxygenase in rat liver after its administration was observed.[12] The present paper describes the results of experiments dealing with the effect of 5-azacytidine on liver serine dehydratase and tyrosine aminotransferase. Increases in enzyme activity were effected by dietary as well as by hormone induction. While the increase of serine dehydratase activity after the administration of tryptophan and cortisone represents synthesis *de novo* of the enzyme,[13,14] the increase of tyrosine aminotransferase following tryptophan administration is not accompanied by the increased rate of formation of this enzyme.

MATERIAL AND METHODS

Material. Holtzman male rats purchased from the Holtzman Rat Company in Madison weighing 200–240 g were used. The intact as well as adrenalectomized animals

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were maintained 3 days on 12·5 per cent protein diet *ad lib.* In all experiments animals were fasted overnight (12–13 hr), and the experiments were begun between 7 and 8 a.m. 5-Azacytidine was synthesized by Dr. A. Píškala (Institute of Organic Chemistry and Biochemistry, Prague). Cortisone acetate was obtained from the Upjohn Company; casein hydrolysate and L-tryptophan were from General Biochemicals. L-Leucine-4,5-^{3}H (45 μc/μmole) was purchased from Schwarz BioResearch, Orangeburg. 5-Azacytidine was injected, i.p., in a maximal volume of 0·5 ml; control animals received the same volume of 0·9% NaCl solution. Intubation was carried out under light ether anesthesia; casein hydrolysate was intubated as a 33 per cent solution (3 ml) and L-tryptophan as a 0·8–1·0 per cent solution, usually 5 ml/animal.

Enzyme assay. At different time intervals after the administration of inducers and/or 5-azacytidine, the groups of animals were sacrificed by decapitation and the livers immediately removed, weighed and homogenized by a Polytron homogenizer in 3 vols. of 0·25 M sucrose in 0·05 M Tris–HCl buffer (pH 7·2) containing 1×10^{-2} M $MgCl_2$, $2{\cdot}5 \times 10^{-2}$ M KCl, 1×10^{-4} M pyridoxal phosphate and 1×10^{-3} M dithiothreitol. Homogenates were centrifuged (28,000 rev/min, 1 hr, 4°) and supernatants stored at −70° within the next 1–2 days. Serine dehydratese and tyrosine aminotransferase were assayed automatically by a combination unit described earlier.[15]

Rate of enzyme synthesis. L-Leucine-4,5-^{3}H was injected, i.p., to groups of 3–6 animals at a dose level of 50 μc/15 nmoles/animal 45 min before killing. High-speed liver supernatant fraction prepared as mentioned above was mixed (usually 0·2 ml) with a rabbit immune serum specific against rat liver serine dehydratase[16] or with a serum specific against rat liver tyrosine aminotransferase (prepared by Dr. E. Baril from McArdle Laboratory for Cancer Research). Total volume was 0·5 ml, and the mixture was incubated for 48 hr at 4°. The antigen–antibody precipitate was centrifuged, washed three times with 3 ml of 0·9% NaCl, and the sediment dissolved in 0·2 ml of formic acid. In all instances, parallel experiments were done utilizing non-immune rabbit serum as controls, and evidence for the completeness of precipitation was obtained.[13,16] The radioactivity was determined in a Packard Tricarb scintillation counter using Scintisol (Isolab) as a counting medium. Incorporation of radioactivity into total proteins of the soluble liver highspeed fraction was measured after precipitation of the proteins with 10% $HClO_4$, repeated washing and delipidization of the precipitate.

RESULTS

Effect of 5-azacytidine on the induction of liver serine dehydratase

The activity of serine dehydratase in the liver depends on the physiological state of the organism and is controlled by the diet.[14,16] The administration of a protein-rich diet or the intubation of casein hydrolysate to rats which have been maintained for several days on 0 per cent protein diet significantly increases the activity of the enzyme.[16] Since the experiments reported here were carried out predominantly with adrenalectomized animals, we used a diet with a lower protein content only (12·5 per cent); the basal activity of serine dehydratase varied around 500–400 μmoles of pyruvate formed/g liver during 1 hr of incubation and not 80–120, a value which can be achieved with the animals kept on a 0 per cent protein diet.[14,16]

The dietary administration of tryptophan results in an increase of serine dehydra-

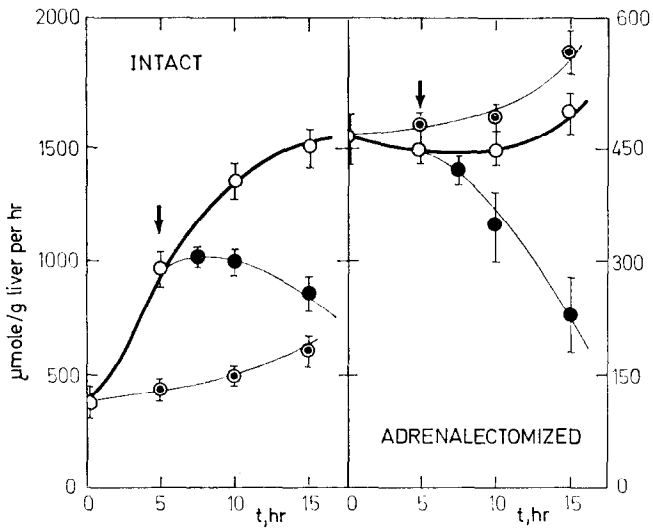

FIG. 1. Different behavior of liver serine dehydratase in intact and adrenalectomized rats after dietary tryptophan and 5-azacytidine administration. Groups of 4–7 rats (200–220 g) kept 3 days on 12·5 per cent protein diet were starved 12 hr before the experiment. L-Tryptophan (20 mg/100 g) was intubated at zero time; 5 hr later (arrow) 5-azacytidine was administered i.p. to groups of intact and adrenalectomized animals (10 and 4 μmoles/100 g respectively), and the activity of enzyme (micromoles per gram of liver per hr) was assayed at different time intervals after tryptophan in the cell-free liver extracts. Activity of the enzyme after inducer only (○——○); after 5-azacytidine (●——●); activity of control animals starved during the experiment (⊙——⊙).

TABLE 1. EFFECT OF DIETARY TRYPTOPHAN ON THE ACTIVITY AND RATE OF THE SYNTHESIS OF SERINE DEHYDRATASE IN THE LIVER OF ADRENALECTOMIZED AND INTACT RATS*

Conditions	Enzyme activity (μmoles/g/hr ±S.E.)	(%)	Enzyme synthesis (dis./min/g ±S.E.)	(%)
Adrenalectomized				
Control	482 ± 93	100	870 ± 54	100
Tryptophan	402 ± 87	83	926 ± 103	106
Tryptophan + 5-AzCR	315 ± 64	65	510 ± 82	59
Intact				
Control	424 ± 53	100	2510 ± 185	100
Tryptophan	960 ± 67	227	6015 ± 728	240
Tryptophan + 5-AzCR	526 ± 84	124	1928 ± 320	77

* Groups of 5–6 adrenalectomized and intact rats (220–230 g) kept 3 days on 12·5 per cent protein diet were starved 12 hr before intubation of L-tryptophan (25 mg/100 g). 5-Azacytidine (5-AzCR) was applied simultaneously with the inducer in a dose level of 4 and 8 μmoles/100 g, respectively, 5 hr before killing. L-Leucine-4,5-^{3}H (50 μc/15 nmoles/animal) was injected, i.p., 45 min before killing.

tase in intact animals only (Fig. 1). The administration of 5-azacytidine leads to the inhibition of dietary-induced serine dehydratase and also to a decrease of the basal enzyme level which increases in starving controls. In Table 1, data are presented on the synthesis of serine dehydratase, which have been obtained by the immunoprecipitation

technique using a specific antibody to purified rat liver serine dehydratase.[14] It is obvious that the rate of enzyme synthesis is increased by the dietary tryptophan in intact animals only. The administration of 5-azacytidine results in a lower pulse-labeling of both the basal serine dehydratase (in the case of adrenalectomized animals) and of the dietary-induced enzyme.

The hormonal induction of serine dehydratase has a different course (Fig. 2). Whereas the increase of enzyme activity in intact animals after the administration of cortisone is insignificant, the hormone induction of the enzyme is very impressive in adrenalectomized rats. Further investigation has led to the finding of different distri-

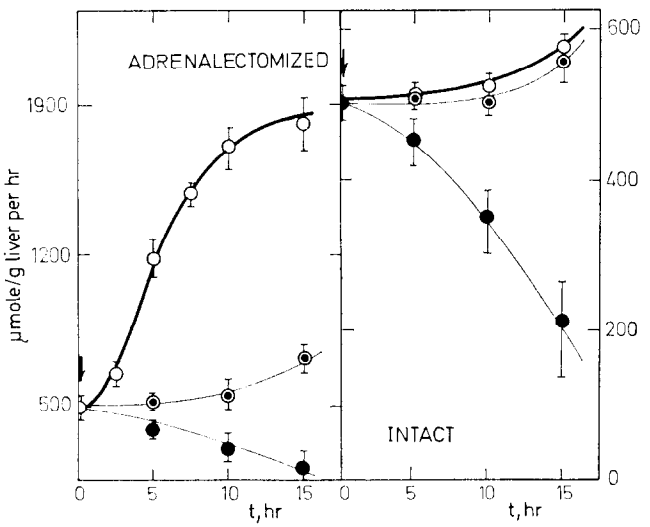

FIG. 2. Effect of 5-azacytidine on cortisone induction of liver serine dehydratase. Groups of 3–6 rats (200–220 g) kept 3 days on 12·5 per cent protein diet were starved 12 hr before the experiment. Cortisone (25 mg/kg) was injected, i.p. at zero time simultaneously with 5-azacytidine to groups of intact and adrenalectomized animals (10 and 4 μmoles/100 g respectively), and the activity of enzyme (micromoles per gram liver per hr) was assayed during the next 15 hr after inducer in the cell-free liver extracts. The arrow indicates the injection of 5-azacytidine (●——●); enzyme activity after cortisone only (○——○); activity of control animals starved during the experiment (⊙——○).

TABLE 2. INHIBITION OF CORTISONE INDUCTION OF SERINE DEHYDRATASE BY 5-AZACYTIDINE*

Conditions	5-Azacytidine (μmole/100 g)	Serine dehydratase		Radioactivity of soluble proteins (10^{-3} × dis./min/g ±S.E.)
		Activity (μmole/g/hr ±S.E.)	Synthesis (dis./min/g ±S.E.)	
Control		475 ± 67	852 ± 43	263·1 ± 12·3
Cortisone	0	1022 ± 120	1845 ± 26	343·5 ± 14·7
Cortisone	3	515 ± 40	667 ± 49	223·1 ± 6·7
Cortisone	6	343 ± 45	375 ± 58	136·0 ± 7·8

* Groups of 6 adrenalectomized rats (240 g) kept 3 days on 12·5 per cent protein diet and starved 12 hr were injected with cortisone (25 mg/kg) simultaneously with 5-azacytidine. The animals were killed 5 hr later. L-Leucine-4,5-^{3}H (50 μc/15 nmoles/animal) was injected, i.p., 45 min before killing.

bution of serine dehydratase isozymes in intact and adrenalectomized animals.[13] As can be seen from Fig. 2, the simultaneous administration of 5-azacytidine decreases the activity of hormone-induced enzyme as well as the level of basal serine dehydratase (in the case of intact animals). The results of the immunoprecipitation experiments shown in Table 2, point to the increased rate of the synthesis of cortisone-induced serine dehydratase; 5-azacytidine decreases the pulse-labeling of the enzyme and the total radioactivity of soluble liver proteins.

Effect of 5-azacytidine on liver tyrosine aminotransferase

Whereas almost exponentially increasing activity of dietary-induced serine dehydratase was blocked by 5-azacytidine administered 5 hr after the inducer (Fig. 1), the activity of tyrosine aminotransferase remained at the same level or even increased,[8,17] depending on the dosage of the drug (Fig. 3). The tryptophan-mediated enhancement of tyrosine aminotransferase activity of the enzyme was observed in its daily rhythm[18] and after the administration of different analogs of nucleic acids, e.g. 5-fluorouracil,[19] 8-azaguanine[20] and 5-fluoroorotic acid.[21,22]

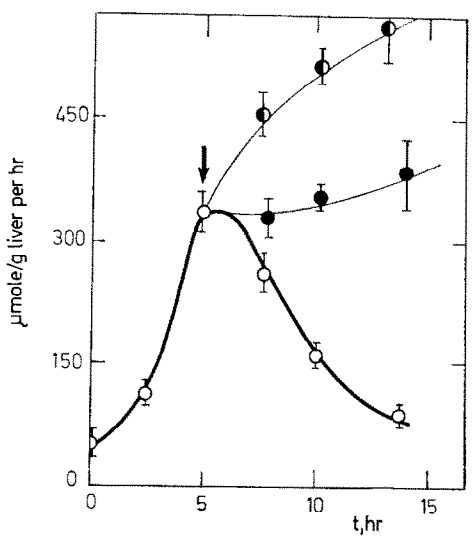

FIG. 3. Modulation of tryptophan-mediated increase of liver tyrosine aminotransferase by 5-azacytidine. Groups of 5–6 adrenalectomized rats (220–230 g) kept 3 days on 12·5 per cent protein diet and starved 12 hr were intubated with L-tryptophan (20 mg/100 g) at zero time. Five hr later 5-azacytidine was injected, i.p., (3 μmoles ◐——◐, and 10 μmoles ●——●/100 g respectively). The enzyme activity (micromoles per gram of liver per hour) was assayed in different time intervals after tryptophan and 5-azacytidine administration (t, hr).

The rate of the synthesis of tyrosine aminotransferase after 5-azacytidine administration was examined by an immunoprecipitation method. Rabbit serum containing a specific antibody against rat liver enzyme was kindly provided by Dr. V. R. Potter. The results of experiments with pulse-labeled tyrosine aminotransferase under various conditions of tryptophan and 5-azacytidine treatment are recorded in Table 3. The

TABLE 3. EFFECT OF DIETARY TRYPTOPHAN AND 5-AZACYTIDINE ON THE ACTIVITY AND RATE OF THE SYNTHESIS OF LIVER TYROSINE AMINOTRANSFERASE*

Conditions	Enzyme activity (μmoles/g/hr ±S.E.)	(%)	Enzyme synthesis (dis./min/g ±S.E.)	(%)
Control	62·5 ± 5·8	100	4310 ± 380	100
Tryptophan (5 hr)	285·4 ± 34·2	456	3870 ± 405	90
Tryptophan (10 hr)	172·1 ± 26·4	275	4125 ± 610	97
Tryptophan (5 hr) + 5-AzCR (5 hr)	398·6 ± 62·5	637	4540 ± 685	105

* Groups of 5–6 adrenalectomized rats (200–220 g) kept 3 days on 12·5 per cent protein diet were starved 12 hr before intubation of L-tryptophan (25 mg/100 g). 5-Azacytidine was applied i.p., 5 hr later in a dose level of 3 μmoles/100 g and 5 hr before killing. L-Leucine-4,5-^{3}H (40 μc/12 nmoles/animal) was injected, i.p. 45 min before killing.

rate of synthesis of the enzyme does not change when tryptophan is given alone or in subsequent combination with 5-azacytidine; in both cases an increase of enzyme activity can be observed (Fig. 3). The possibility of dilution of leucine-^{3}H which is used for pulse-labeling of the enzyme after the administration of 5-azacytidine was eliminated experimentally.

The cortisone induction of tyrosine aminotransferase, which takes place both in adrenalectomized and intact animals, is paralleled by an increased synthesis *de novo* of the enzyme (Table 4). Unlike the tryptophan-mediated enhancement of tyrosine

TABLE 4. INHIBITION OF CORTISONE INDUCTION OF TYROSINE AMINOTRANSFERASE BY 5-AZACYTIDINE*

Conditions	5-Azacytidine (μmole/100 g)	Tyrosine aminotransferase: Activity (μmole/g/hr ±S.E.)	Tyrosine aminotransferase: Synthesis (dis./min/g ±S.E.)	Radioactivity of soluble proteins (10^{-3} × dis./min/g ±S.E.)
Control		64·1 ± 7·2	3720 ± 470	306·5 ± 28·2
Cortisone	0	627·3 ± 42·1	8820 ± 790	381·2 ± 24·7
Cortisone	3	442·7 ± 39·3	5055 ± 610	274·3 ± 18·9
Cortisone	6	310·6 ± 62·0	3315 ± 470	165·7 ± 9·8

* Groups of 5–7 adrenalectomized rats (230 g) kept 3 days on 12·5 per cent protein diet and starved 12 hr were injected, i.p., with cortisone (25 mg/kg) simultaneously with 5-azacytidine 5 hr before killing. L-Leucine-4,5-^{3}H (50 μc/15 nmoles/animal) was injected, i.p., 45 min before killing.

aminotransferase (Table 3), the cortisone induction of the enzyme is in agreement with the increased rate of its synthesis *de novo*. Simultaneous administration of 5-azacytidine and the inducer results in the decreased synthesis of the enzyme and in a decrease of its activity. However, the decline of tyrosine aminotransferase activity is less marked than of serine dehydratase (Table 2).

As obvious from Fig. 4, the activity of tyrosine aminotransferase is either decreased or increased after the administration of 5-azacytidine, depending on the duration of the time interval after the administration of the drug. Five hr after simultaneous administration of 5-azacytidine and cortisone, the enzyme activity is lower than in

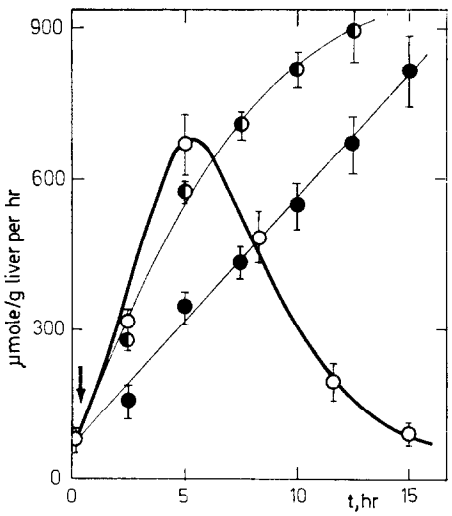

FIG. 4. Tyrosine aminotransferase activity in the liver of rats treated with cortisone alone and in combination with 5-azacytidine. Groups of 4–6 adrenalectomized rats (220–240 g) kept 3 days on 12·5 per cent protein diet and starved 12 hr were injected, i.p., with cortisone (25 mg/kg) alone (○——○) or in combination with 5-azacytidine (3 μmoles ◐——◐, and 8 μmoles ●——●/100 g, respectively). The enzyme activity (micromoles per gram of liver per hour) was assayed in different time intervals after the inducer (t, hr).

the control, cortisone-induced animals, and synthesis of the enzyme is also inhibited (Table 4). During longer time intervals after the administration of 5-azacytidine, when injected cortisone no longer leads to an additional increase of tyrosine aminotransferase, the activity of the enzyme is higher in animals which have simultaneously received the drug (Fig. 4). The increase of enzyme activity is no longer paralleled by its increased synthesis *de novo*.

DISCUSSION

The studies on the induction of liver enzymes in the presence of 5-azacytidine have provided a new insight into the biological action of this drug.[3,12,17,21] In our opinion, the interference of 5-azacytidine with the liver pyrimidine synthesis *de novo*,[6] the block of ribosomal biosynthesis[23] and the degradation of liver polyribosomes[8,11,17] cannot explain completely the biological activity of the drug. In addition to the inhibition of cortisone induction of tryptophan oxygenase,[12] a significant inhibitory effect of 5-azacytidine has been observed in hepatic ornithine decarboxylase,[3] in dietary-induced ornithine aminotransferase and histidine ammonia lyase (unpublished observations). The data presented in Figs. 1 and 2 indicate that also the activity of dietary and hormone-induced serine dehydratase is completely blocked after the administration of the drug. The enhancement of enzyme activity is associated with the increased rate of enzyme synthesis; 5-azacytidine decreases both the activity of the enzyme and its synthesis *de novo* (Tables 1 and 2).

Tyrosine aminotransferase and uridine kinase, the activity of which also increases in 5-azacytidine-treated animals,[24] are obviously controlled by different regulatory mechanism. (Cycloheximide possesses the same effect as 5-azacytidine as was demonstrated earlier in tyrosine aminotransferase[25–27] and recently in uridine kinase.[28]) The effect of 5-azacytidine on the tryptophan-mediated enhancement of tyrosine

aminotransferase (Fig. 3 and Table 3) suggests that synthesis or activity of the responsible degrading system is more sensitive to the effect of 5-azacytidine than the synthesis of tyrosine aminotransferase itself. The administration of 5-azacytidine results in a slow increase in the activity of tyrosine aminotransferase in non-induced rat liver; the rate of its synthesis remains unaltered (unpublished observation). If the system responsible for the degradation of tyrosine aminotransferase were sensitive to 5-azacytidine, then the administration of the drug would result in the increase of the enzyme activity under conditions of a nearly constant rate of its synthesis.

It is generally accepted that the inactivation of tyrosine aminotransferase after its hormonal induction depends on the synthesis of the degrading enzyme system.[20] The simultaneous administration of 5-azacytidine and hormone leads to a partial inhibition of tyrosine aminotransferase activity as well as of its synthesis (Table 4). Actinomycin D shows a similar effect both in intact animals[19] and in HTC cells in tissue culture.[29] When given after maximum induction of tyrosine aminotransferase, an additional rise in enzyme activity (superinduction) was observed although the same dose of the drug blocks the induction if given to cells with or before steroid. The evidence was presented that superinduction was due to the increase in tyrosine aminotransferase synthesis and not to any alteration in enzyme decay.[29] The stimulatory effect of actinomycin D on tyrosine aminotransferase has been confirmed in Reuber H35 hepatoma cell line.[30] However, the obtained data indicate that actinomycin D-mediated increase in tyrosine aminotransferase activity is due to a specific blocking decay of the enzyme while its synthesis continues. The data given in Tables 3 and 4 speak in favor of a similar interference of 5-azacytidine with the degradation of liver tyrosine aminotransferase.

The finding that 5-azacytidine produces neither superinduction nor interferes with actinomycin D-mediated induction of tyrosine aminotransferase in HTC cells[29] is not in accordance with our observations. The increase of tyrosine aminotransferase after 5-azacytidine administration, shown in Figs. 3 and 4, is in agreement with the data and conclusion of Levitan and Webb[17] indicating that the maintenance of tyrosine aminotransferase activity at the induced level by 5-azacytidine results from a failure to re-initiate the degradation of the enzyme. However, differential sensitivity of the induction of tyrosine aminotransferase to 5-azacytidine in the liver and Hepatoma 5123 D exists, and can be attributed to differences in controls operating at the post-transcriptional level.[3]

The increased activity of tyrosine aminotransferase[8,21] and uridine kinase[24] as well as the enhancement of liver RNA synthesis[7] after 5-azacytidine administration indicate that the drug interferes either with the activity and/or turnover of degrading systems in the liver or with their respective enzyme repressors. However, the specific site of this attack has not been elucidated up to now. On the other hand, the administration of 5-azacytidine is associated with the impaired synthesis of serine dehydratase, and equally of tyrosine aminotransferase when the drug is given simultaneously with the inducer. Thus 5-azacytidine besides interfering with degrading enzymes affects directly the formation of liver enzymes under the condition of hormone and dietary induction.

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