for curative action. Thus, the drug sensitivity of the strain is totally different from the ones studied so far, which offers the possibility to reveal agents with a different strain specificity than the known ones.

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Deletion of Serine Dehydrase and Cystathionine Synthetase Activities During Azo-Dye Carcinogenesis. (26476)

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The Novikoff hepatoma, a tumor induced in rat liver using 4-dimethylaminazobenzene (DAB)(1), was reported to be essentially devoid of serine dehydrase activity(2). Recently, Selim and Greenberg(3) reported that a purified protein from rat liver exhibited both serine dehydrase and cystathionine synthetase activities. The authors suggested that their data indicated both activities were exhibited by a single enzyme(3). Assuming this suggestion to be correct, and further assuming that the enzymatic constitution of transplanted lesions of hepatic origin represented multiple deletions during hepatocarcinogenesis(4,5), it was reasonable to expect that cystathionine synthetase would also be essentially absent in the Novikoff hepatoma. It was the purpose of this investigation to determine (a) whether both serine dehydrase and cystathionine synthetase activities were absent in several transplanted hepatomas and (b) whether both enzyme activities were altered during hepatocarcinogenesis with 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB).

Materials and methods. The manner in which transplanted hepatomas were carried, precancerous liver tissues were obtained, and primary lesions were induced with 3'-Me-DAB have been previously described(6-8). Pyruvic acid formation, determined by the method of Friedemann and Haugen(9), was used as the criterion for assaying serine dehydrase activity of tissue homogenates, while serine utilization, determined by the periodate oxidation method of Frisell *et al.*(10), was used to assay cystathionine synthetase activity. The chromogen formed from chromotropic acid and formaldehyde was measured at 570 mμ and compared to an appropriate standard curve constructed with L-serine. All optical density measurements were made using a Bausch and Lomb Spectronic 20 Colorimeter.

Direct estimation of cystathionine was achieved by one dimensional paper chromatography(11) using n-butanol, acetic acid and water (60:15:25) as the solvent system. Known compounds and supernatants obtained by centrifugation of reaction mixtures treated with 10% trichloroacetic acid (TCA) were spotted on 47 cm × 57 cm sheets of Whatman No. 3 paper and subjected to descending solvent flow. Following partition,

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color was developed using 0.25% ninhydrin in acetone(12).

Livers from 3 animals were finely minced with scissors and washed with 1.2% postassium chloride (KCl) containing 5×10^{-3} M ethylenediaminetetraacetate (EDTA). Sufficient tissue (wet weight) was added to KCl-EDTA solutions to yield a 40% homogenate. Tumor tissues were freed of gross necrotic areas and connective tissue elements and were homogenized in a similar manner. The Novikoff ascites cells were occasionally exposed to 15 second sonic oscillation to ensure complete cell disruption.

Cystathionine synthetase activity was determined by adding 1 ml of the 40% homogenate to 3 ml of a reaction mixture containing 0.04 M DL-homocysteine†, 0.033 M L-serine, 3.2×10^{-3} M EDTA, 5×10^{-5} M pyridoxal phosphate and 1.3×10^{-3} M 2,3-dimercaptopropanol. The total volume of the incubation mixture was brought to 6 ml by addition of 2 ml of 0.2 M borate buffer, pH 8.5. Reaction mixtures were incubated 1 hour at 38°C under 95% nitrogen 5% CO_2. Synthetase activity was determined by the amount of serine utilized with appropriate corrections for non-enzymatic serine utilization and conversion to pyruvate. The unit of cystathionine synthetase activity was defined as μmoles serine utilized/mg protein/60 minutes incubation. Protein was determined by the method of Lowry *et al.*(13).

Serine dehydrase activity was assayed by diluting 40% homogenates with KCl-EDTA to yield an 8% homogenate (wet weight/volume) and 1 ml of the diluted homogenate was added to the incubation mixture. The incubation mixture was identical with that used for assay of cystathionine synthetase except that DL-homocysteine was deleted. Total volume of reaction mixtures was 3 ml and incubation period was 20 minutes at 38°C under 95% nitrogen 5%CO_2. The unit of serine dehydrase activity was defined as μmoles pyruvate produced/mg protein/20 minutes incubation. Under conditions described, linear pyruvate formation was observed through 30 minutes incubation and protein concentrations of 4-6 mg/ml through 20 minutes. Cystathionine synthetase activity yielded linear reaction velocities for 90 minutes and a protein concentration of 10-14 mg/ml for 60 minutes incubation. Experiments in which the pH of both systems was varied indicated agreement with the reported optimum of 8.2-8.3(3).

Results. Serine dehydrase and cystathionine synthetase activities of rat liver, transplanted hepatomas and primary hepatomas were determined. The data are shown in Table I. None of the hepatomas, either transplanted or primary, had detectable amounts of either serine dehydrase or cystathionine synthetase activity. On the other hand, both enzyme activities were readily demonstrated in normal liver, livers from animals maintained on the semi-synthetic diet and liver tissue adjacent to primary lesions. When tumor homogenate was added to liver homogenate no significant alteration in either enzyme activity was observed. Furthermore, neither enzyme activity was activated in tumor homogenates by addition of liver homogenates or a boiled extract of liver homogenate.

Since the assay for cystathionine synthetase activity was dependent upon estimating the amount of serine utilized, further validation of cystathionine synthetase activity seemed advisable. When TCA extracts of incubation mixtures were subjected to paper chromatography(11) data as shown in Table II were obtained. Although liver homogenates consistently yielded evidence of cystathionine synthesis, no evidence of synthesis was observed with any of the transplanted hepatomas. Thus, these data (a) confirmed the previous observation that serine dehydrase was absent in the Novikoff hepatoma (2) and extended this observation to include other tumors of hepatic origin, and (b) showed that tissues lacking dehydrase activ-

† Sources were as follows: L-Serine, H. and M. Chemical Co.; Pyridoxal phosphate, Sigma Chemical Co.; DL-Homocysteine thiolactone, California Corporation for Biochemical Research. The thiolactone was converted to DL-homocysteine by adjusting an aqueous solution to pH 8.8 with 3 N NaOH just prior to its addition to the enzyme assay reaction mixture.

TABLE I. Serine Dehydrase and Cystathionine Synthetase Activities of Normal Rat Liver, Transplanted Hepatomas and Primary Hepatomas.

Tissue	Enzyme activity* Serine dehydrase (units)		Cystathionine synthetase (units)	
Liver†	(9)‡	1.01 ± .10§	(5)	.30 ± .05
" ‖	(3)	.43 ± .04	(3)	.25 ± .18
Novikoff hepatoma	(3)	0	(4)	0
" " (ascites form)	(3)	0	(3)	0
3'-Me-DAB hepatoma	(3)	0	(3)	0
Primary "	(3)	0	(3)	0
Liver tissue adjacent to primary lesions	(3)	.52 ± .06	(3)	.28 ± .03

Conditions: For enzyme assays see text.

* Units: Serine dehydrase, μmoles pyruvic acid formed/mg protein/20 min.; cystathionine synthetase, μmoles serine utilized/mg protein/60 min.

† Animals maintained on Rockland chow diet.

‡ No. of experiments. For each experiment tissues from 2 to 3 animals were pooled.

§ Stand. dev.

‖ Animals maintained on semi-synthetic, riboflavin-deficient diet for 12 wk.

ity also lacked cystathionine synthetase activity.

The absence of either serine dehydrase activity or cystathionine synthetase activity in primary hepatomas (Table I) indicated the loss of these enzyme activities during hepatocarcinogenesis with 3'-Me-DAB. Therefore, it was important to determine whether these losses occurred in precancerous liver. Activity of these enzymes in livers of animals fed 3'-Me-DAB for 0, 4, 8 and 12 weeks was compared to the activity of animals ingesting the basal diet (Table III). After 4 weeks on 3'-Me-DAB both serine dehydrase and cystathionine synthetase activity dropped sharply, however, by the 8th week activity had returned to normal or higher levels. At 12 weeks values approximated those observed in basal diet animals and values observed for liver tissue adjacent to primary lesions. Sub-

TABLE II. Paper Chromatography of Reaction Mixtures for Assay of Cystathionine synthetase Activity.*

			Position of ninhydrin positive compounds† R_f values	
L-cystathionine		(4)‡	.098 ± .003§	
L-homocysteine		(2)		.197 ± .006
L-serine		(3)		.203 ± .004
Reaction mixture for cystathionine synthetase activity with:				
Liver homogenate	No incubation	(2)		.205 ± .020
	60 min. incubation	(2)	.096 ± .019	.210 ± .025
Novikoff ascites homogenate	No incubation	(2)		.216 ± .008
	60 min. incubation	(2)		.216 ± .019
Novikoff homogenate	No incubation	(2)		.204 ± .021
	60 min. incubation	(4)		.205 ± .013
3'-Me-DAB hepatoma homogenate	No incubation	(2)		.204 ± .021
	60 min. incubation	(2)		.215 ± .020

* Solvent system, n-butanol : acetic acid : water (60:15:25).

† Color developed by .25% w/v ninhydrin in acetone.

‡ No. of experiments.

§ Stand. dev.

TABLE III. Comparison of Serine Dehydrase and Cystathionine Synthetase Activities of Rat Liver during Carcinogenesis with 3'-Methyl-4-dimethylaminoazobenzene.

	Enzyme activity*			
	Serine dehydrase		Cystathionine synthetase	
Time, wk	Basal	3'-Me-DAB	Basal	3'-Me-DAB
0	(3)† .60 ± .08‡		(3) .26 ± .09	(3)
4	(3) .42 ± .06	(3) .28 ± .12	(3) .16 ± .12	(3) .02 ± .41
8	(3) .40 ± .08	(3) .96 ± .27	(3) .19 ± .09	(3) .27 ± .12
12	(3) .43 ± .04	(3) .42 ± .03	(3) .25 ± .18	(3) .26 ± .04

Conditions: For enzyme assays see text. All animals were conditioned on basal diet 7 days prior to start of experiment. 3'-Me-DAB concentration was 0.06%.

* Units: Serine dehydrase, μmoles pyruvate formed/mg protein/20 min. incubation; cystathionine synthetase, μmoles serine utilized/mg protein/60 min. incubation.

† No. of experiments. For each experiment tissues from animals were pooled.

‡ Stand. dev.

sequent experiments in which 4'-fluoro-DAB‡, DAB, 2'-methyl-DAB or 4'-methyl-DAB were fed to animals for 4 weeks, activity losses with both enzymes approximated those observed at 4 weeks with 3'-Me-DAB. Since these azo dyes differed markedly in their ability to induce lesions(14), it appeared that the early losses in both enzyme activities were not associated with carcinogenesis *per se*.

Throughout the present study, under conditions which led to alteration or loss of serine dehydrase activity, a similar alteration or loss of cystathionine activity was observed. Although these similarities could have been due to other factors, the data supported the contention that the conversion of serine to pyruvate and the condensation of serine and homocysteine to form cystathionine were reactions catalyzed by a single enzyme(3).

Since conversion of homocysteine to cysteine is mediated by cystathionine synthetase(3) and cystathionase(15), absence of cystathionine synthetase activity may account for the inability of homocysteine to replace cystine in the *in vitro* growth of the 3'-Me-DAB hepatoma(16). A similar inability to replace the cysteine requirement with homocysteine was reported for chick embryonic heart fibroblast cultures(17), however, Eagle (18) reported that in cultures of human cell strains, homocysteine was utilized for cysteine synthesis. It was subsequently shown (19) that the apparent homocysteine-cysteine interconversion was due to mobilization of cysteine residues bound to the serum component of the medium.

‡ DAB refers to 4-dimethylaminoazobenzene.

Summary. Serine dehydrase and cystathionine synthetase activities were determined in rat liver, precancerous livers, primary hepatomas induced with 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) and transplanted hepatomas induced with azo dyes. Both enzyme activities were absent in all malignant tissues assayed. After 4 weeks on diets containing 3'-Me-DAB both enzyme activities were reduced more than 50% but returned to normal values at 8 and 12 weeks. Precancerous alterations in these enzyme activities did not appear to be correlated with the carcinogenic activity of azo dyes employed. Deletion or alteration patterns in all tissues assayed supported the contention that both enzyme activities were manifested by a single apoenzyme.

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Histamine Release into the Circulation by Meperidine (Demerol®).* (26477)

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Hypotension is the primary cardiovascular effect of meperidine. This pharmacologic action has been demonstrated in the dog with various intravenous doses, both small and large. This fall in blood pressure has been attributed to a number of mechanisms, including vasodilatation by direct action, central vasomotor depression and perhaps, ganglionic blockade. Schacter(1) measured increases in free histamine in cat muscle and skin after treatment with meperidine. Subsequently, the possibility that histamine release contributes in part to the mechanism of meperidine induced hypotension has been suggested by several workers(2,3). Since there is no quantitative information concerning this effect in the intact dog, this study was done to find out if this drug is a histamine releasing agent in the dog and if so, whether enough histamine is released to cause hypotension.

Methods. Anesthetized (pentobarbital, 35 mg/kg) mongrel dogs received artificial ventilation through a cuffed endotracheal tube by means of a Starling pump. Blood pressure was recorded continuously from a catheter in the femoral artery connected to a Statham pressure transducer. The tracing was obtained on an Offner direct writing recorder. After a steady state was obtained, meperidine 5 mg/kg of a 10% solution, was injected rapidly into the femoral vein, followed with 1 ml of isotonic saline. Prior to and at one, 3 and 5 minutes after injection, samples of venous blood were taken into siliconized syringes containing 1:100 of 10% disodiumethylenediaminetetraacetate. Histamine was measured by the method of Shore and co-workers(4) with one modification; a Farrand photofluorometer, model A was employed instead of a spectrophotofluorometer. The approximate activating and fluorescent wave lengths were isolated by use of Corning glass filters, primary no. 5860 and secondary no. 3389. The sacrifice of some selectivity was replaced by the gain in ease of measuring duplicate specimens. Quenching occurred at a lower final concentration of histamine but the latter was proportional to fluorescence over the range .005 μg/ml to .165 μg/ml. All measurements were performed on duplicate specimens. Three dogs received intravenous injections of histamine, .03 mg/kg. The dose was calculated from the average increase in blood histamine found at the one minute interval after meperidine injection in 5 dogs. Blood histamine was measured in only one of the 3 dogs.

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