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Phenanthrene in Relation to Growth of Rats Maintained on a Low-Casein Diet.

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It was shown that halogenated benzenes and naphthalene, when fed to growing rats which were maintained on a diet low in casein but sufficient for moderate growth, check the growth. Upon incorporation of cystine, methionine or glutathione into the diet which contained either halogenated benzenes or naphthalene, the growth was resumed. These results were interpreted as indicating that the halogenated benzenes and naphthalene induced cystine deficiency in the rat which was alleviated by the administration of the sulfur-containing amino acids. This interpretation was supported by the fact that the halogenated benzenes and naphthalene are known to yield in the rat the corresponding mercapturic acids.[1, 2]

Some time ago evidence was presented to the effect that phenanthrene, when fed to dogs, might be excreted in the urine as a mercapturic acid.[3] It appeared of interest to investigate the effect of phenanthrene on growth of rats which were kept under the experimental conditions referred to above.[1, 2] Should the effect of phenanthrene on growth of rats be similar to that produced by halogenated benzenes and naphthalene it would, perhaps, be justifiable to assume that phenanthrene, like anthracene,[4] is detoxicated in the rat to yield, in part, a mercapturic acid. The present study was therefore undertaken.

The general experimental procedure used in the present study was similar to that employed previously.[1, 2] Two litters of albino rats, 30 days old, were used. The composition of the diet (referred to in the table as Diet C-6) was as follows: Casein, 6 parts; corn starch, 50; sucrose, 15; Osborne and Mendel[5] salt mixture, 4; and Crisco, 25. In addition to the diet each rat received daily 400 mg of dry yeast and 100 mg of cod liver oil. Phenanthrene was fed in doses of 0.500 g per 100 g of the diet. 0.500 g of l-cystine, 0.640 g of dl-methionine, or 0.500 g of taurine per 100 g of the diet was fed as

1 White, A., and Jackson, R. W., *J. Biol. Chem.*, 1935, **111**, 507.

2 Stekol, J. A., *J. Biol. Chem.*, 1937, **121**, 87; 1937-38, **122**, 55.

3 Stekol, J. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 170.

4 Boyland, E., and Levi, A. A., *Biochem. J.*, 1936, **30**, 1225.

5 Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 572.

supplements to the diet which contained phenanthrene. For the sake of economy of space the data are presented in summarized form in Table I.

TABLE I.
Effect of Phenanthrene on Growth of Rats Fed Diet C-6 With or Without l-cystine, dl-methionine, or taurine.*

Rat	Food intake per day g	Supplement per 100 g diet g	Phenanthrene per 100 g diet g	Days on diet	Initial wt, g	Total gain, g
1 ♂	6.6	0	0	40	48	+33
	5.1	0	0.5	14	81	— 5
	7.4	0.5 cystine	0.5	14	76	+16
	6.0	0	0.5	14	92	— 2
	7.7	0.64 methionine	0.5	14	90	+12
2 ♀	6.2	0	0	40	44	+39
	4.8	0	0.5	14	83	— 9
	6.8	0.5 cystine	0.5	14	74	+15
	5.5	0	0.5	14	89	— 5
	7.4	0.64 methionine	0.5	14	84	+16
	7.7	0	0	14	100	+12
3 ♂	5.8	0	0	40	49	+32
	5.0	0	0.5	14	81	— 5
	4.8	0.5 taurine	0.5	14	76	— 7
	7.8	0.64 methionine	0.5	14	69	+16
	6.6	0	0.5	14	85	— 2
	7.2	0.5 cystine	0.5	14	83	+14
	7.5	0	0	14	97	+10
4 ♀	5.7	0	0	40	46	+40
	5.5	0	0.5	14	86	— 6
	6.6	0.5 cystine	0.5	14	80	+10
	6.0	0.5 taurine	0.5	14	90	— 2
	7.0	0	0	14	88	+14
	6.3	0	0.5	14	102	— 5
	7.9	0.64 methionine	0.5	14	97	+12

* This data for each rat presented in this table are representative of experiments obtained on 4 animals.

The results presented indicate that phenanthrene, when fed to growing rats, checks the growth. The growth is resumed upon the administration of l-cystine, dl-methionine but not taurine. In this respect phenanthrene is similar to halogenated benzenes or naphthalene. Pending the isolation of detoxication products of phenanthrene from the urine of rats which were fed the hydrocarbon, it is assumed that the cessation of growth of rats on phenanthrene diet is induced by the depletion of cystine reserves by phenanthrene for detoxication purposes. Apparently the detoxication of phenanthrene in the growing rat takes precedence over the needs of the animal for growth purposes.

Summary. 1. Phenanthrene was fed to growing rats which were

maintained on a low-casein diet. The growth ceased. It was resumed upon incorporation, into the phenanthrene diet, of l-cystine, dl-methionine but not taurine. 2. Assumption is made that phenanthrene is detoxicated in the rat to yield a mercapturic acid, the formation of which is responsible, at least in part, for the cessation of growth of rats ingesting phenanthrene.

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Identity of "Inhibitor" and Antibody in Extracts of Virus-Induced Rabbit Papillomas.

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Saline extracts of the virus-induced papillomas of domestic rabbits often contain something that inhibits or neutralizes the virus *in vitro*, as Shope first noted.[1] Serological studies have shown that when mixed with it the blood of rabbits carrying these papillomas also has in most instances the power to neutralize the virus *in vitro*.[2] Experiments were undertaken to learn whether the "inhibitor" procured from the papillomas may not be specific antiviral antibody of the sort present in the blood.

To test for "inhibitor", 10% extracts of freshly procured papillomas were prepared by grinding in sand, suspending in saline, and centrifuging at about 4400 rpm for 20 minutes in an angle-head centrifuge. The clear supernatant fluids were then mixed in equal parts with a Berkefeld filtrate of the virus-induced growths of cottontail rabbits, containing virus of known titer, incubated 2 hours at 37°C, and rubbed into scarified areas on the skin of normal domestic rabbits according to a titration technic already described.[2]

The papillomas of 13 domestic rabbits with high serum-antibody titers, as determined by virus-neutralization and complement-fixation tests,[2, 3] all yielded large amounts of the "inhibitor", extracts of the growths neutralizing completely or almost completely an amount of virus equal to 500 minimal infective doses. Similar growths of 6 domestic rabbits which had but little circulating antibody yielded

1 Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

2 Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79.

3 Kidd, J. G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.