Table 4. Components of variance (exclusive of the between-sexes variance) as percentage variance.

Source of variance	Phenotypic variance	Heritability	Genetic component	Environmental component
Between groups	60.4	0.42	25.4	35.0
Within groups	39.6	0.13	5.1	34.5
Total	100	0.30	30.5	69.5
(Between sexes)	(7.9)	(1)	(7.9)	

0.30; this is of similar order to that at the beginning of the experiment, when it was tested and found to be 0.25.

The experiment has therefore confirmed the various points that were under test. Intergroup mobility dependent upon a variable does lead to genetic differences between groups even under conditions where there are strong environmental differences between groups. In fact, in the particular conditions of this experiment the genetic and environmental differences between groups are not of very different importance. On the other hand, despite the environmental difference, much of the genetic variation has sorted out *between* groups at the expense of within-group variance.

The human situation is much more complex than that in our experiment. For example, there is intra- as well as interclass social heredity in the human situation, whereas in our experiment the parent-offspring environmental correlation is entirely between groups; the correlation between human social mobility and any particular variable is incomplete, whereas we have tried to make it complete in our experiment; and the environmental difference between human classes is complex and doubtless very heterogeneous, whereas in our experiment we have made the controlled environmental difference between groups simple and have made it correlate completely with groups. Further, we stress that no importance should be attached to the actual heritabilities or components of variance obtained in our experiment, for they would have differed had we used a smaller or greater temperature difference, a base stock with different initial heritability, or one showing genotype-environment interaction with respect to temperature. It is therefore obvious that extrapolation from our experiment, or indeed from any other animal experiment, must be made only with extreme caution.

Nevertheless, we do feel that our experiment is relevant to the human situation inasmuch as it strengthens the expectation that social mobility related to a heritable variable will give rise to some genetic difference between class means despite strong parent-offspring environmental correlation. We therefore believe that our experimental results support those who hold the view that neither cultural nor genetic approaches alone are likely to lead to adequate explanations of social class phenomena.

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3 December 1969

Bladder Cancer Induction by Aromatic Amines: Role of N-Hydroxy Metabolites

Abstract. *Repeated installation of* N-*hydroxy-2 naphthylamine into dog bladders produced tumors of this organ. There is a correlation of degree of carcinogenicity of 1-naphthylamine, 2-naphthylamine, and 4-aminobiphenyl with both methemoglobin production in the blood and concentration of* N-*oxidation products in the urine. Thus,* N-*hydroxylation appears to be the key to bladder cancer production by aromatic amines.*

After the discovery of the role of *N*-hydroxylation in hepatocarcinogenesis by 2-acetylaminofluorene (*1*), the search for the urinary metabolite active in the induction of bladder cancer by 2-naphthylamine in man and the dog has centered on *N*-hydroxy-2-naphthylamine. The discovery of this substance in the urine of dogs that were given 2-naphthylamine supported this concept (*2*). However, the demonstration of the occurrence of *N*-hydroxy-1-naphthylamine in the urine of dogs that were given large doses of 1-naphthylamine (*3*), coupled with the observation that *N*-hydroxy-1-naphthylamine is more carcinogenic than *N*-hydroxy-2-naphthylamine when tested by intraperitoneal administration to rats (*4*), casts serious doubt on the relevance of *N*-hydroxylation to the induction of bladder cancer.

In an attempt to resolve the dilemma, we found that in the dog the *N*-hydroxynaphthylamines are further oxidized to nitroso compounds (Fig. 1). With thin-layer and gas chromatography, we found both 1- and 2-nitrosonaphthalene in urine. Gas chromatography is a rapid and sensitive way to determine these substances in urine. After the administration of 2-naphthylamine to dogs, the urine, collected by catheter, was adjusted to *p*H 4. Ferricyanide was added to convert the *N*-hydroxy-2-naphthylamine to 2-nitrosonaphthalene. The urine was then extracted with petroleum ether, and the extract was injected on the gas chromatograph. The peak obtained, therefore, represented the sum of *N*-hydroxy and nitroso compounds present which we refer to as total *N*-oxidation products. Ferricyanide could not be used in the measurement of the *N*-oxidation products of 1-naphthylamine. Adjustment of the *p*H to 6.5, however, allowed the extraction with petroleum ether of both *N*-hydroxy and nitroso metabolites. *N*-hydroxy-1-naphthylamine is quantitatively converted to 1-nitrosonaphthalene on the column at 125°C. Studies of mixtures of *N*-hydroxy and nitroso derivatives of both amines added to urine were performed. Recoveries in the range of 80 to 100 percent were obtained. Sensitivity of detection on the electron-capture detector was 100 pg of these substances, and thus we were able to measure 40 parts per billion in 5 ml of urine. This procedure was also applicable to the determination of the *N*-hydroxy metabolites of 4-aminobiphenyl, a bladder carcinogen recently shown in this laboratory to be distinctly more potent than 2-naphthylamine (*5*).

The urine of dogs that had been given the maximum acute dose of these amines that they could tolerate (70 mg/kg) was examined for *N*-oxidation products by gas chromatog-

raphy. No significant difference was observed between 2-naphthylamine and 1-naphthylamine. However, when the dose was reduced to 5 mg/kg, a dose at which 2-naphthylamine is carcinogenic and 1-naphthylamine is not, negligible quantities of *N*-oxidation products were found in dogs given 1-naphthylamine and considerable quantities of *N*-oxidation products (0.2 percent of the administered dose) were found in dogs given 2-naphthylamine (Fig. 2). The same dogs given 5 mg of 4-aminobiphenyl per kilogram of body weight produced yet higher amounts of *N*-oxidation products averaging about 1.5 percent of the administered dose.

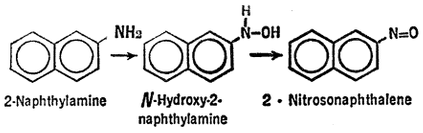

Fig. 1. Oxidation of 2-naphthylamine.

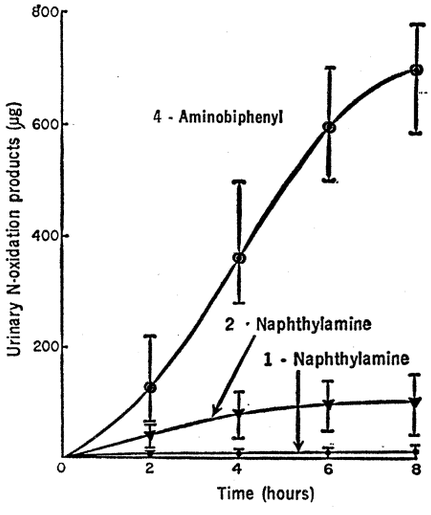

Fig. 2. Mean cumulative excretion of total *N*-oxidation products of four beagle dogs given single doses (5 mg/kg) of 1-naphthylamine, 2-naphthylamine, or 4-aminobiphenyl. Ranges indicated.

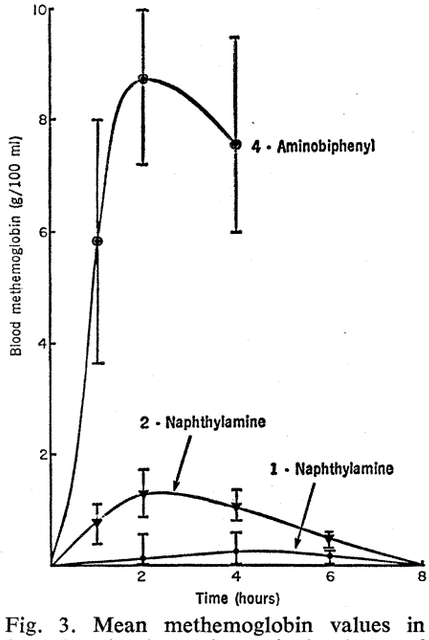

Fig. 3. Mean methemoglobin values in four beagle dogs given single doses of 1-naphthylamine, 2-naphthylamine (70 mg/kg), or 4-aminobiphenyl (25 mg/kg). Ranges indicated.

We believe that the *N*-hydroxy derivatives of aromatic amines are responsible for the methemoglobinemia commonly observed in man and experimental animals exposed to these substances (*6*). In working with large doses of 1-naphthylamine, 2-naphthylamine, and 4-aminobiphenyl in dogs, it became apparent that the most potent carcinogen, 4-aminobiphenyl, produces severe methemoglobinemia more readily than the other two substances. When the production of methemoglobinemia by 1- and 2-naphthylamine was compared quantitatively at a dose of 70 mg/kg, methemoglobin concentrations in the dogs given 2-naphthylamine reached a mean of 1.3 g/100 ml and concentrations in the dogs given 1-naphthylamine were consistently lower (Fig. 3). A dose of 70 mg of 4-aminobiphenyl per kilogram of body weight was rapidly fatal to dogs; and even when the dose was reduced to 25 mg/kg, three of the four dogs died. The maximum methemoglobin levels observed at this dose (Fig. 3) ranged between 7.2 and 10.3 g/100 ml.

When interest first began to develop in the role of *N*-hydroxylation in bladder cancer, we initiated an experiment to test the carcinogenicity of *N*-hydroxy-2-naphthylamine by direct repeated application to the dog bladder mucosa. A dimethylsulfoxide solution of *N*-hydroxy-2-naphthylamine (5 mg in 5 ml) was instilled by catheter into two male and two female beagles twice a month for 30 months. When these animals were killed 45 months after the first instillation, three had bladder tumors. Similar groups of four dogs each treated with 2-naphthylamine in dimethylsulfoxide or dimethylsulfoxide alone developed no tumors.

Thus the case for the role of *N*-hydroxylation in the production of bladder cancer by aromatic amines is complete. A correlation has been established between the carcinogenicity of these three aromatic amines, their methemoglobin-producing capabilities (a measure of *N*-hydroxylation in the blood), and the amount of *N*-hydroxy metabolites in the urine. Although the noncarcinogen 1-naphthylamine is *N*-hydroxylated, and the *N*-hydroxy metabolite is carcinogenic, negligible amounts occur in the urine at the doses usually given to dogs, or to which humans would be exposed. It, therefore, seems clear that *N*-hydroxy-2-naphthylamine or perhaps the closely related nitroso derivative is the active urinary carcinogens.

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Beta Glucuronidase Activity in Skin Components of Children with Cystic Fibrosis

Abstract. *As compared to that in normal children a decreased activity of beta glucuronidase, a lysosomal enzyme associated with mucopolysaccharide metabolism and with salt transport, has been detected in the epidermis and sweat gland tissues of children with cystic fibrosis.*

We have found a decreased activity of beta glucuronidase in certain skin components in children with cystic fibrosis. Cystic fibrosis is an inherited disease characterized by excretion of an abnormally viscous mucus from various exocrine glands and by excretion of abnormally high concentrations of salt in the sweat (*1*). The disease usually produces pulmonary infection and exocrine pancreatic deficiency and is usually fatal.

Beta glucuronidase is a lysosomal enzyme widely distributed in animal tissues. Linker *et al.* (*2*) observed that this enzyme along with other hydrolytic enzymes, degrades acid mucopolysaccharide fragments derived from hyal-