Effect of Carbon Blacks on Carcinogenic Compounds.*† (23951)

E. VON HAAM, H. L. TITUS, IRVING CAPLAN, AND GEORGE Y. SHINOWARA
Department of Pathology, Ohio State University, Columbus

Von Haam(1) reported briefly on production of 6 epitheliomas in a total of 212 animals with benzene extracts of carbon blacks employed in the rubber industry: The active fractions were obtained by Scholl and Brock employing a procedure based on chromatographic separation and differential solubility. Subsequently, it was suggested that the apparent carcinogenic activity would probably be insignificant in the original carbon blacks because the isolated substances would be not only too low in concentration, but also firmly adsorbed(2). At about the same time Falk and Steiner(3) isolated 3,4 benzpyrene, 1,2 benzypyrene, pyrene and chrysene from benzene extracts of rubber stoppers and one old automobile tire. In later reports(4,5) they stated that benzene extracts of some carbon blacks contained small quantities of 3,4 benzpyrene, 1,2 benzpyrene and 5 other related aromatic hydrocarbons. They found a rough correlation between amounts of extractable aromatic hydrocarbons and average particle diameter of the carbon blacks and suggested that the difference in extractable aromatic hydrocarbons from carbon blacks may be explained by the phenomenon of adsorption. Despite the demonstrated presence of benzpyrene traces in some carbon blacks by two independent laboratories, Ingalls(6) found no evidence of environmental cancer among carbon black workers. In this communication are the results of *in vitro* and *in vivo* investigations on 7 carbon black preparations and their effect on carcinogenicity of 20-methylcholanthrene, 3,4 benzpyrene and p-dimethylaminoazobenzene.

Methods and materials. Adsorption procedure. Exactly 200 mg amounts of the carcinogenic substance in saturated cyclohexane solution were added to 20, 10, 5, 2.5, 0.625 and 0.313 g carbon black. The volumes were each brought up to 150 ml with cyclohexane. Each suspension was shaken mechanically for 2 hours and then filtered through a #1 Whatman paper into a 2 liter volumetric flask. The filter residues were washed with cyclohexane up to volume. The optical densities of the resulting filtrates were obtained at 360 mμ for 20-methylcholanthrene, 385 mμ for 3,4 benzpyrene and 410 mμ for p-dimethylaminoazobenzene: C.P. cyclohexane was the optical reference and 1.0 cm quartz cells were used throughout. Optical densities were also obtained on filtrates obtained by the identical procedure, except that the carcinogen was omitted. Moreover, for each series of unknowns, the optical densities of standard solutions of carcinogenic substances were also determined. *In vivo experiments.* Harlan stock rats and albino Swiss mice were used. The animals were kept in air-conditioned rooms and were fed a standard diet of Purina Dog Checkers with weekly additions of carrots and green vegetables except those in the azo dye experiments who received a pure rice diet since this has been shown to be most effective in production of neoplasms(7). For each carcinogenic substance, identical dosages were given in the free and adsorbed states. In addition an equivalent amount of carbon black without the carcinogenic substances was also administered. Thus, 3,4 benzpyrene was applied by painting the skin of mice twice weekly with acetone solution of the carcinogen at 0.5% concentration to one group; an acetone suspension of the filter residue containing the carcinogen adsorbed to carbon black to a second group; and a pure carbon black suspension in the same solvent to a third group. In another series of experiments, 20-methylcholanthrene either in the unadsorbed or the adsorbed state was injected subcutaneously into each rat in an approximate amount of 2 mg suspended in 1 ml of olive oil(8). P-dimethylaminoazobenzene was mixed with polished rice to give an approximate concen-

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TABLE I. Adsorption of Carcinogenic Compounds by Various Carbon Blacks.

Carbon black					mg carcinogen adsorbed by 1 g carbon black		
	Properties						
Designation	Total surface*	E.M. surface†	Diameter, mμ	pH	20-methyl-cholanthrene	3,4 benz-pyrene	p-dimethyl-aminoazo-benzene
NeoSpectra Mark II	40	22	13	3.0	111.8	86.4	47.5
Peerless	20	12	24	2.8	40.4	41.3	27.5
Micronex-deactivated	10	10	29	10.5	17.0	12.2	9.5
Kosmos 60	11	11	27	8.9	10.9	11.7	6.7
Micronex beads	10	10	29	4.5	9.6	8.8	7.2
Philblack 0	10	10	29	9.0	9.7	9.7	4.9
Micronex	10	10	29	4.5	8.3	7.5	3.3

* Total surface values in acres/lb are based on iodine adsorption measurements.
† E.M. surface values in acres/lb are based on electron microscope measurements, assuming smooth particle surfaces.

tration of .06% and the animals were fed *ad libitum*. The azo dye adsorbed to carbon black was mixed with food to produce an equal concentration (0.06%). All the animals were sacrificed at the end of the experiment and a complete autopsy was performed on each animal in order to determine any effect the carbon black itself had upon the organism. The application sites as well as all organs including the central nervous system were examined histologically.

Results. Table I lists the mg methylcholanthrene, p-dimethylaminoazobenzene and benzpyrene adsorbed by 1 g carbon black as related to the physical properties of the 7 carbon blacks. It is apparent that NeoSpectra Mark II has the highest adsorption property followed by Peerless and Micronex deactivated. The least adsorption was found with Micronex. As far as the carcinogenic compounds are concerned, the amounts of 3,4 benzpyrene and 20-methylcholanthrene were almost equal and approximately twice that of p-dimethylaminoazobenzene. Because of the low adsorption of azobenzene to certain carbon blacks, the data on Philblack O and Micronex are estimations at best since they were obtained by considerable extensions of experimental curves. Moreover, the optical densities of the filtrates plus washings of all 7 carbon blacks in the control experiments were 0.00 or identical with that of C.P. cyclohexane, indicating the absence of substances absorbing light at 360, 385 and 410 mμ.

The results obtained from the *in vivo* adsorption study with 20-methylcholanthrene are summarized in Table II. In the positive control group using 20-methylcholanthrene in olive oil, 54% of the animals showed tumors 4 to 8 months after initiation of the experiment. The tumors proved to be highly undifferentiated spindle-cell sarcomas. The control animals injected with carbon black (Philblack O, 210 mg; Kosmos 60, 185 mg; Micronex Beads, 240 mg) did not develop any tumor in 12 months. Animals injected with 20-methylcholanthrene adsorbed on these carbon blacks developed tumors approximately at the same time as the control animals. Those injected with 20-methylcholanthrene adsorbed to Micronex Beads, developed tumors much later. Since these data suggested that adsorption to carbon blacks does not interfere appreciably with the carcinogenicity of 20-methylcholanthrene with the possible exception of that adsorbed to Micronex Beads, an additional experiment was carried out in which this carcinogen adsorbed to carbon black in 20% olive oil suspension was allowed to stand at room temperature in a sealed container: In 4 weeks, approximately one-fifth of the carcinogen was eluted by the olive oil.

The data from the *in vivo* experiments with p-dimethylaminoazobenzene are also presented in Table II. The nearly perfect inhibitory effect of adsorption on the carcinogenicity of butter yellow is broken only by a single hepatoma which appeared 10¼ months after the beginning of the experiment in one animal fed butter yellow adsorbed to Philblack O. None of the other animals have shown any evidence of neoplasm in spite of the common occurrence of liver cirrhosis. The

TABLE II. Effect of Carbon Blacks on Carcinogenicity of 20-Methylcholanthrene, p-Dimethylaminoazobenzene and 3,4 Benzpyrene.

Substances administered			Results		
				Interval (mo)	
Carcinogen	Carbon black	Total No. animals	% tumors	First tumor	50% of tumors
Methylcholanthrene	None	24	54	4	6½
None	Philblack 0	24	0	0	0
Methylcholanthrene	*Idem*	24	29	4	6½
None	Kosmos 60	24	0	0	0
Methylcholanthrene	*Idem*	24	66	3½	5
None	Micronex beads	24	0	0	0
Methylcholanthrene	*Idem*	24	16	7	8½
Azobenzene	None	24	58	6	9
None	Philblack 0	24	0	0	0
Azobenzene	*Idem*	24	4	10¼	—
None	Micronex	24	0	0	0
Azobenzene	*Idem*	24	0	0	0
None	Kosmos 60	24	0	0	0
Azobenzene	*Idem*	24	0	0	0
Benzpyrene	None	48	96	2	3½
None	NeoSpectra Mark II	48	0	0	0
Benzpyrene	*Idem*	48	69	3	5¼
None	Peerless	48	0	0	0
Benzpyrene	*Idem*	48	63	3	6
None	Kosmos 60	48	0	0	0
Benzpyrene	*Idem*	48	59	3½	6
None	Micronex	48	0	0	0
Benzpyrene	*Idem*	48	54	3½	5
None	Micronex-deactivated	48	0	0	0
Benzpyrene	*Idem*	48	54	3¼	5

number of survivors in all but the positive control series is still considerable (42%) 15 months after initiation of the experiment. The animals ate without difficulty food containing the following concentrations of carbon black: Philblack O, 12%; Micronex, 18%; and Kosmos 60, 9%.

The effect of carbon black adsorption on carcinogenicity of 3,4 benzpyrene is summarized in Table II. In the positive control group 96% of the animals developed fatal carcinoma. None of the 240 animals which served as carbon black controls developed any tumor: The acetone suspensions contained from 6 to 60% carbon black. Of all the 240 animals treated with carbon black-adsorbed 3,4 benzpyrene suspended in a solvent, 54 to 69% developed tumors, the earliest of which appeared 3 months after the beginning of the experiment, a delay of one month as compared with the positive controls. Duration of the benzpyrene experiments was 9 months and number of survivors ranged from 33 to 57%. Analyses of the supernate obtained from the acetone suspended material demonstrated that 10.0-23.4% of the adsorbed compound was eluted by this solvent and therefore acted as free 3,4 benzpyrene. In an attempt to circumvent this problem of elution, the adsorbed 3,4 benzpyrene without solvent was rubbed on the skin of 72 mice twice weekly: At the end of 24 months, no tumors were observed in this group as well as in 72 mice receiving carbon black alone. However, in 24 positive control mice treated with the dry carcinogen alone, only 12% of the animals developed tumors in contrast to 96% when 0.5% benzpyrene in acetone was employed.

Discussion. The results of the physical experiments conclusively demonstrate that carbon blacks adsorb 3 well-known carcinogenic compounds and that degree of adsorption depends on type of carbon black. There was a close parallelism between total surface values of the various carbon blacks and amounts of

20-methylcholanthrene, 3,4 benzpyrene and p-dimethylaminoazobenzene adsorbed. It would also appear from examination of the data obtained on the 3 varieties of Micronex that excessively high pH, such as 10.5, is another factor. The 2 pentacyclic hydrocarbons had twice the affinity to carbon blacks than the azo compound.

The data indicate that adsorption to carbon blacks has a varied but definite inhibitory effect on the carcinogenicity of the 3 compounds investigated. The almost complete reduction in the carcinogenic activity of azo dye by carbon blacks was just as pronounced as that reported by Hanszen and Selle(9). These investigators fed montmorillinite adsorbed dye to 20 animals for 175 days and only one developed a tumor as compared to 18 of the 20 control rats. The effect of the various carbon blacks on the carcinogenicity of 20-methylcholanthrene varied widely. Micronex Beads significantly reduced the number of tumors and delayed their appearance while Kosmos 60 was not inhibitory. A probable explanation for the latter results is associated with the finding that the vehicle, olive oil, elutes 20-methylcholanthrene which had been adsorbed on carbon black. Similarly, elution must also be considered in the 3,4-benzpyrene experiments, in which although the number of tumors decreased and their appearance delayed there was incomplete inhibition of carcinogenicity by carbon black. The acetone eluted material had a concentration of 0.10 to 0.23% free 3,4 benzpyrene, which according to Crabtree(10) can be carcinogenic.

Another interesting fact arising from these experiments is that carbon black, *per se*, either fed, painted with an acetone suspension or injected subcutaneously in olive oil suspension has not produced a single benign or malignant tumor. It must be noted that the carcinogenic substances or activity previously reported(1-4) were found in fractions obtained by exhaustive extraction, separation and concentration procedures on carbon black alone or on carbon black containing products such as rubber stoppers or tires.

Summary. 1. Adsorption of 20-methylcholanthrene, 3,4 benzpyrene and p-dimethylaminoazobenzene to 7 different carbon blacks has been determined quantitatively. Differences in the amount adsorbed are related not only to the specific carcinogen but also to the total surface value of a carbon black. 2. Administration of carbon black alone by feeding, painting and subcutaneous injection did not produce any benign or malignant tumor in 460 animals. 3. The carcinogenicity of p-dimethylaminoazobenzene was almost completely inhibited by carbon black. Only 1 out of 72 animals fed carbon black adsorbed azo dye developed a hepatoma. 4. Administration of carbon black adsorbed 20-methylcholanthrene and 3,4-benzpyrene in combination with olive oil and acetone respectively inhibited carcinogenesis to a lesser degree. The incidence of tumors occurring in these experiments was approximately that expected from the concentrations of the free carcinogen eluted by the vehicle from the carbon black. 5. It is therefore suggested that in the adsorbed state all 3 compounds tested are not carcinogenic and that carcinogenicity in two of the compounds was restored due to elution by either olive oil or acetone.

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