formed, in each of which 2- to 3-day-old flies were collected as separate batches representing successive hatchings from a set of culture bottles. In both experiments extracts of females were found to contain twice the amount of methionine detected in extracts of males. With one exception the amounts found in the separate batches of a single experiment were relatively constant for each sex. However, the amounts of methionine found in the second experiment were higher in both sexes. The amounts of free methionine found are among the highest so far reported to have been found in an animal tissue (*9*). Separate studies have eliminated the possibility that the difference in males and females is attributable to the contents of the gut. The possibility that males and females have a differential ability to metabolize methionine is now under investigation.

WILLIAM D. KAPLAN
JOSEPH T. HOLDEN
BENJAMIN HOCHMAN*
Departments of Genetics and
Biochemistry, Medical Research Institute, City of Hope Medical Center, Duarte, California

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* Present address: University of Utah, Salt Lake City.

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Elution of 3,4-Benzpyrene and Related Hydrocarbons from Soots by Plasma Proteins

The carcinogenic polycyclic aromatic hydrocarbon, 3,4-benzpyrene, has been identified as a constituent of soot. Its action in inducing skin cancers has been explained on the basis of the lipid solvent action of sebaceous secretions in eluting 3,4-benzpyrene from soot particles. Renewed interest in the carcinogenic properties of soot has followed the epidemiologic observation that the risk of development of lung cancer is greater among urban residents than among rural residents. Any description of the role soot may possess in the pathogenesis of human lung cancer must include an explanation for its biological activity in the lung.

In an earlier study we demonstrated that the carcinogenic hydrocarbon, 3,4-benzpyrene, which is routinely demonstrably adsorbed on soot, is absent in soot-laden human lungs (*1*). The morphologic demonstration of the intracellular entry of soot particles of appropriate size made it advisable to determine the effect of cellular and plasma proteins on the elution of benzpyrene from soot.

Soots of two representative sizes were studied in vitro for quantitative hydrocarbon determinations. One soot, of an average particle size of 80 mμ, was freed of essentially all polycyclic aromatic hydrocarbons by appropriate extraction methods and enriched by the adsorption of 3,4-benzpyrene onto the particles in a ratio of 1 μg of 3,4-benzpyrene to 1 mg of soot. The second soot was a commercial carbon black with an average particle size of 500 mμ. In their natural state these large particles contain a number of adsorbed polycyclic hydrocarbons.

The soots of the two sizes were handled in essentially the same manner. Ten-milligram samples of the enriched soot and 50- and 100-mg samples of the large-sized soot were placed in contact with an aqueous test medium consisting of 25 or 50 ml of sterile human plasma and were incubated at 37°C. The solutions were shaken constantly for 90 minutes in the 1.5- and 16-hour experiments and for 5-minute intervals to a total of 60 minutes of shaking in the remaining experiments. Incubation periods varied from 3 hours to 8 days. After exposure of the soot to the test medium, centrifugation or filtration was used to separate the soot from plasma. Three or four extractions with hot acetone were necessary for the removal of the adsorbed hydrocarbons from the soot.

Table 1. Percentage recovery of polycyclic aromatic hydrocarbons from 500 mμ soot and plasma after incubation of various durations; *t* trace; *p*, present.

Compound (μg/100 mg of soot)	1.5 hours			16 hours			96 hours			192 hours			Saline soot
	Soot	Plasma	Total	Soot	Plasma	Total	Soot	Plasma	Total	Soot	Plasma	Total	
Pyrene (8.4)	9	81	90	34	61	95	9	50	59	6	61	67	100
Fluoranthene (1.0)	*t*	≈ 100		*p*	*p*		0	*p*		*p*	*p*		100
Compound X (2.8)	0	100	100	*t*	93		0	*p*		0	39	39	100
1,2-Benzpyrene (2.9)	*t*	52		17	41	58	0	21	21	*t*	21		100
3,4-Benzpyrene (1.7)	18	82	100	41	59	100	6	23	29	18	18	36	100
1,12-Benzperylene (9.2)	31	66	97	67	33	100	5	15	20	11	13	24	100
Anthanthrene (2.4)	25	54	79	54	33	87	*t*	17		13	13	26	100
Coronene (10.0)	40	36	76	66	26	92	12	8	20	15	10	25	100

Although separation of the enriched small-sized soot from plasma was laborious, analysis for but a single carcinogenic hydrocarbon made the procedure relatively simple. In marked contrast, separation of large-sized soot from plasma was readily accomplished; however, analysis was more complicated because of the presence of several polycyclic aromatic hydrocarbons. In all instances, chromatography on a short column of activated alumina was necessary before accurate qantitation was possible.

Analysis for 3,4-benzpyrene was carried out with a Beckman DK-2 spectrophotometer. Fluorescence spectroscopy was used for the differentiation of 3,4-benzpyrene and 1,12-benzperylene. The plasma phase was similarly extracted, with ether as the solvent, and 3,4-benzpyrene quantitation was undertaken by the chromatographic and spectrophotometric methods described above. Control experiments were identical to the test ones except for the substitution of saline as the medium in the former.

After exposure to plasma proteins, the 3,4-benzpyrene remaining on the small-particle soot decreased slowly. After 3 days of incubation, 60 percent of the benzpyrene remained on the soot; and after 8 days, the quantity of 3,4-benzpyrene present dropped to 35 percent.

The elution pattern of the various aromatic polycyclic hydrocarbons from the large-sized carbon black (500 mμ) by plasma could readily be determined. Both the amounts eluted by plasma and the amounts retained on soot for the polycyclic aromatic hydrocarbons studied are shown in Table 1. Of the eight hydrocarbon compounds detected by the spectrophotometric technique, the following were found to be readily extracted by plasma: pyrene, fluoranthene, compound X, and 1,2-benzpyrene. The remaining identified polycyclic aromatic hydrocarbons in soot—3,4-benzpyrene, 1,12-benzperylene, anthanthrene, and coronene—were eluted at a lesser rate. Complete retention of 3,4-benzpyrene on the

soot particles was noted in the control experiments when saline was substituted for plasma.

In the course of incubations carried out for periods of from 3 to 192 hours, it was demonstrated that the degree of elution of polycyclic aromatic hydrocarbons from soot by plasma paralleled the elution of these compounds by nonpolar solvents (petroleum ether and ether) from activated alumina.

The carcinogenic implications of the elution of 3,4-benzpyrene from soot by plasma proteins warrant review. In urban air, pollutants have been shown to inhibit ciliary action so that abnormal deposition and retention of soot particles occur. Phagocytosis of soot particles occurs, and the elution of benzpyrene from the soot by the intracellular proteins results in abnormally high local concentration of desorbed polycyclic aromatic hydrocarbons, including 3,4-benzpyrene. An environment favorable to the biological activity of the carcinogenic hydrocarbons results.

HANS L. FALK
ADELE MILLER
PAUL KOTIN

Departments of Pathology and *Biochemistry, University of Southern California School of Medicine, Los Angeles,* and *Department of Pathology, Los Angeles County General Hospital*

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Tests for Digestion of Algal Polysaccharides by Some Marine Herbivores

Although algae form the primary diet of many marine animals and digestion of some components of algae in the gut is apparent from an examination of the changes undergone during passage in the gut, it is of interest to know whether the enzymes of the gut, or of the glands attached to it, digest the algal constituents. Purification of some of the algal carbohydrates (*1*) has made it possible to study digestion of these substances by extracts of the gut wall. The gut extracts of three herbivorous forms in Monterey Bay were therefore tested for their ability to digest several algal carbohydrates.

In each case the gut, thoroughly washed with sterile sea water, was ground with sand in chilled buffer at a *p*H equivalent to that found in the gut as well as at *p*H values slightly to either side of it. After incubation of the extract and carbohydrate (under toluene) for a period of time which varied from 4 or 5 hours to 1 day, the quantity of reducing sugar released during digestion was tested by the Somogyi method (*2*). In all cases the blank, consisting of the sum of the reducing sugar of gut extract in buffer and of a mixture of the test carbohydrate in buffer, was subtracted from the reducing sugar found in the mixture of carbohydrate, extract, and buffer in which digestion was occurring. Because the rate of digestion is affected by temperature, preliminary tests were made to ascertain the optimal conditions for digestion when digestion of a substance occurred readily, and the same conditions were then provided in other cases.

Intestinal extract of the purple sea urchin, *Strongylocentrotus purpuratus,* proved incapable of digesting laminarin and fucoidin, although previous experiments (*3*) indicated its ability to digest iridophycin. A bacterial suspension from the gut of the urchin, however, digests the entire algae and their various constituents.

Puggetia producta, the kelp crab, while apparently omnivorous, eats algae in aquaria, when starved. Tests with extracts of its digestive gland showed it to have a potent amylase which quickly digests starch and glycogen but has no action on laminarin, fucoidin, agar agar, carragheenin, and a *Gigartina* polysaccharide. Failure to find positive results in these cases might have been due to improperly designed experiments, but the positive results obtained with *Cryptochiton* extracts on some of these polysaccharides, when similar techniques were used, makes it seem probable that the crab does not digest these constituents by virtue of its own enzymes if, indeed, these constituents are digested in its gut at all.

In the giant chiton, *Cryptochiton stelleri,* the digestive tract extract was found to have a very active amylase which readily digests starch and glycogen (*p*H 5.8 for the "stomach" extract and *p*H 6.8 for extract of the intestine). Tests showed that the chiton stomach extract digested the algal polysaccharides laminarin and fucoidin and that the extract of the intestine affected the first but not the second; digestion proceeded at a reasonable rate, comparable to that for digestion of starch and glycogen in some cases, somewhat more slowly in others. Since the action of the intestinal extract was less marked, tests with it were discontinued. However, the extracts of stomach were ineffective on cellulose, agar agar, iridophycin, carragheenin, and sodium alginate.

It is therefore possible that many of the algal constituents are not digested by herbivores and are passed out as part of the "roughage" of the diet. Certainly, when some marine herbivores are abundantly supplied with food in aquaria, they pass feces which contain many portions of ingested algae still colored and intact (*3*). They may therefore be able to use only the more readily available materials such as protein and floridean starch (in red algae), which are present in small amounts, necessitating intake of a large bulk of algae. However, young growing tips of algae contain a large percentage of proteins (*4*), and after feeding of proteins to some marine forms, the content of nonprotein nitrogen in their body fluid rises markedly (*3, 5*). On the other hand, it is also possible that bacteria in the gut of many marine herbivores digest constituents of the algae which the herbivore is unable to digest. While microbes capable of digesting various algal polysaccharides can be isolated from the gut and are sometimes present in large numbers (*3, 6*), they may occur as contaminants, and it is difficult to be certain that they perform this role. Even bacteria in the vertebrate rumen are able to digest some algal polysaccharides (*7*).

HELEN HUANG
ARTHUR C. GIESE

Department of Biological Sciences, Stanford University, Stanford, California

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