Effect of Halogenated Aromatic Hydrocarbons on Proteins of Rat Tissues. (22779)

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The object of the present work is to study the effect of administration of various halogenated hydrocarbons on the protein content of rat tissues. Previous work(1-9) has indicated that the tissues are the most probable source of cysteine for the formation of mercapturic acids which are formed in the rat from various aromatic hydrocarbons. In view of the continuous exchange and renewal of tissue proteins at the expense of dietary amino acids, it appeared possible that the ingestion of aromatic hydrocarbons by the rat will interfere with the protein formation in the tissues by the interaction of the sulfhydryl groups with the administered hydrocarbons. The blocking of the sulfhydryl groups by the hydrocarbons should be reflected not only in the decrease in the protein content of the tissues but also in the decrease of the sulfhydryl and disulfide content of the proteins.

Methods. Albino rats of Sprague-Dawley strain were born and raised in the laboratory. Young rats, 30 to 38 days of age, weighed 63 to 133 g, older rats were of 86 to 186 days of age, weighing 176 to 366 g. In each case, age, sex, and weight of experimental and control rats were the same. Animals were housed in individual metabolism cages and water allowed *ad libitum*. Weights of animals and food consumption were recorded twice a week. Diet "D" had the following per cent composition: vitamin-free casein 8; sucrose 15; salt mixture 4 (U.S.P. XIV); Crisco 20; cod liver oil 5; corn starch 48. 200 mg of choline chloride and a complete mixture of all known vitamins in adequate amounts were mixed with the diet. Diet "A" contained 27% of casein and 29% of starch, the rest of the ingredients were in the same concentration as in Diet "D." The nitrogen content of both diets was determined. *Halogenated aromatic hydrocarbons.* Phenacyl bromide, p-chlorobromobenzene, benzyl chloride, and naphthalene tetrachloride were commercial products, and the purity of the products was established in the laboratory. As previously recorded by others(7), large amounts of the compounds sharply reduced food intakes, caused a rapid loss in weight, leading eventually to death of the animals. On autopsy, pulmonary hyperemia, hemorrhagic kidneys and occasionally hemorrhages in the pericardium were observed. No other abnormal histological changes in the lungs or kidneys were observed. The quantities of hydrocarbons fed to rats were reduced to amounts which could be tolerated by the animals. With the intraperitoneal injections of naphthalene tetrachloride, the dose was 100 mg (as a water suspension containing 1% "Tween 80")/100 g weight. *Analytical methods.* The nitrogen content of diets, excreta, and various organs was determined by the Conway ultramicro Kjeldahl diffusion method. In nitrogen balance studies the urine collected daily, and feces, collected separately, were analyzed twice a week. All determinations were made in at least triplicate. The results of nitrogen balance studies were charted according to Reifenstein *et al.*(10). The protein content of tissues was calculated as suggested by Addis *et al.*(11,12). In each tissue water content and nitrogen, the latter in a homogenate, were determined. Whole blood was used for the nitrogen analysis. Animals were anesthesized under ether, blood samples were taken from abdominal aorta, and organs removed and thoroughly washed of all blood. Organs of rats from the same experimental groups were pooled, and analyses made on pooled samples. The sulfhydryl and disulfide groups in blood were determined in the formic acid-hydrochloric acid digests employing the procedure of Kolb and Toennies(13). Total circulating blood volume was determined with T-1824 (Evans-Blue). The ratio of muscle to body weight had been previously determined on 12 adult male rats, with

TABLE I. Effect of Halogenated Aromatic Hydrocarbons in Growing Rat.*

No. and sex of rats	Hydrocarbon, % in diet	Intake diet	Days	Avg intake per day, g	Avg daily change, g
6 ♀	Phenacyl bromide, 0.5	D	38	5	−.85
6 ♀	p-Chlorobromobenzene, 1.0	D	52	8	.28
6 ♀	None	D	45	10	1.16
12 ♂	N. T., 0.25	D	30	6	−.70
12 ♂	None	D	30	6	.47
12 ♂	N. T., 1.0	A	30	21	3.46
12 ♂	None	A	30	21	5.60
6 ♀	N. T. in glycerin, 0.5	A	13	12	1.53
6 ♀	None	A	13	12	2.81
6 ♀	N. T. in glycerin, 0.25	D	19	7	−.84
6 ♀	None	D	19	7	1.15

* "N. T." denotes naphthalene tetrachloride. Intramusc. injections of N. T. emulsions in water or olive oil into rats fed diets D or A did not significantly affect growth rates, due, perhaps, to poor absorption of the hydrocarbon from site of inj.

a mean of 41%, the coefficient of variation $\left(\frac{\text{Standard Deviation}}{\text{Mean}}\right)$ being 10.5%. Weight of skeleton was calculated from body weight using the data of Donaldson(14).

Results. The data in Table I show that phenacyl bromide, p-chlorobromobenzene, or naphthalene tetrachloride inhibited growth of rats which ingested the 8% casein diet. Similar results, not reported here, were obtained on mice which were fed benzyl chloride, p-chlorobromobenzene, bromobenzene, or naphthalene. No significant inhibition of growth was obtained in young rats which ingested the same hydrocarbons with the 27% casein diet, although some inhibition of growth was observed in rats which were administered naphthalene tetrachloride as glycerin emulsion. The results in Table II show that the

TABLE II. Response of Adult Rats to Naphthalene Tetrachloride.

No. of rats	Naphthalene tetrachloride	Diet	Intake days	Avg daily intake	Avg daily wt change, g
6	0.25% in diet	D	58	10	−1.15
6	*Idem*	D*	10	14	4.55
5	"	D	58	12	.58
5	"	D*	10	13	2.40
6	1% in water, intraper.	A	15	10	−7.51
6	None	A	16	20	2.71
6	In glycerin, intraper.	A	17		−1.05
6	None	A	43		.62

* 0.12% of L-cystine in diet.

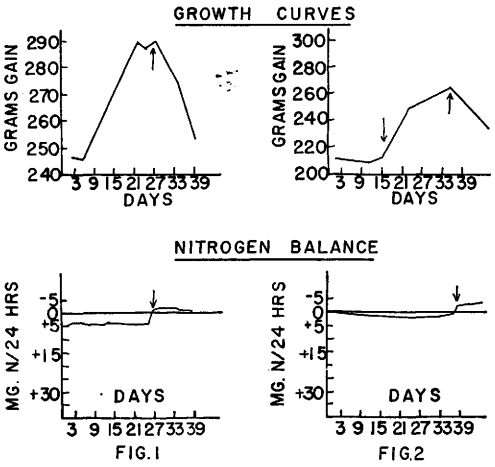

FIG. 1 and 2. Effect of naphthalene tetrachloride on gain in wt and nitrogen balance in rats. Data represent avg results obtained on 6 male rats 128-132 days old. Upper curves are wt curves; lower curves represent nitrogen balance. Animals fed same diet for 14 days before collection of excreta. Animals represented by left 2 curves were fed Diet A plus 0.25% naphthalene tetrachloride. Arrow indicates time at which animals were continued to be fed Diet A but naphthalene tetrachloride was inj. intraper. The right 2 curves represent animals which were fed Diet D plus 0.25% naphthalene tetrachloride. First arrow indicates time at which L-cystine was added to diet. Second arrow indicates time at which naphthalene tetrachloride was inj. intraper. to rats while feeding of Diet D was continued.

intraperitoneal injection of naphthalene tetrachloride into adult rats produced significant loss in weight, even when the animals ingested the 27% casein diet. The results on nitrogen balance in these animals are summarized in Fig. 1 and 2. The animals re-

TABLE III. Protein of Tissues of Rats Which Received Naphthalene Tetrachloride Intraperitoneally for 12 Days, Calculated in g per Rat per 310 cm² of Body Surface.*

	Group 1 Diet A	Group 3 Diet A + N.T.	Change in protein content, %	Group 2 Diet D	Group 4 Diet D + N.T.	Change in protein content, %
Liver	1.57	1.49	− 5	1.41	1.56	11
Kidneys	.26	.22	−14	.22	.24	9
Digestive tract	.88	.50	−42	.72	.67	− 7
Muscle	13.85	8.40	−39	12.87	10.89	−13
Lungs	.13	.11	−15	.10	.13	30
Spleen	.07	.04	−41	.05	.04	−20
Heart	.11	.08	−27	.12	.09	−25
Blood	1.99	1.22	−38	1.69	1.26	−25
Pelt	8.02	7.09	−11	6.21	4.25	−31
Skeleton	1.55	1.34	−13	1.46	1.42	− 3
Carcass	7.88	5.57	−29	7.30	5.56	−23
Total	36.31	26.06	−23	32.13	26.10	−19

* The data were obtained on pooled tissues of animals in each group. Nitrogen balance of these animals during the 12 days of N. T. inj. is indicated in Fig. 1 and 2 by the respective arrows at extreme right portion of figures.

mained in positive nitrogen balance while ingesting diet "A" or "D" with naphthalene tetrachloride. However, when naphthalene tetrachloride was injected a definite negative nitrogen balance was observed. After 12 days of injection of naphthalene tetrachloride the animals were sacrificed, the organs were analyzed for protein content, and the results are shown in Table III. It will be noted that a loss of protein has occurred in all tissues examined, except in animals which ingested diet "D." In the latter case an increase in the protein content of the liver, kidneys, and lungs was noted. The data in Table IV suggest that a decrease in the sulfhydryl and particularly in the disulfide content of the rat blood has occurred during the injection of naphthalene tetrachloride.

Discussion. Loss in weight of rats ingesting hydrocarbons together with the 8% casein diet does not appear to be due entirely to the drop in food consumption, as was indicated by paired feeding experiments. The prompt resumption of growth on incorporation of L-cystine into the hydrocarbon-containing diet suggests interference of the hydrocarbon with the utilization of cystine (cysteine). This inference is supported by the apparent decrease in the -SH and S-S content of the blood during the intraperitoneal administration of naphthalene tetrachloride. It is of interest to note that Tsuji(15) and Nakashima(16) observed a decrease in the free and "bound" cysteine of the eye lens proteins as well as of glutathione of the liver and eye lens of rabbits which were fed naphthalene. As has been established, naphthalene undergoes *in vivo* conjugation with cysteine and is in part excreted in the urine of animals as the naphthalene mercapturic acid. It is possible that naphthalene tetrachloride is similarly metabolized in part to a mercapturic acid derivative. The data further suggest that in the course of metabolic disposal of the hydrocarbons examined an interference with a normal metabolism of proteins in various tissues takes place which is minimized either by a high protein content of the diet or by supplementary cystine. Binding of various hydrocarbons to the tissue proteins has been emphasized in recent years. We have no direct evidence that the hydrocarbons which we examined in this study combine directly with the tissue pro-

TABLE IV. Sulfhydryl and Disulfide Content of Blood of Rats Which Receive Intraperitoneally Naphthalene Tetrachloride for 12 Days.*

Diet and hydrocarbon	Sulfhydryl for 310 cm² of original body surface	Disulfide for 310 cm² of original body surface
A	1.36	19.52
A + N. T.	.68	5.22
D	1.12	12.93
D + N. T.	.71	6.75

* Sulfhydryl and disulfide expressed in mg as cysteine and cystine respectively/rat.

teins, although the possibility exists that the hydrocarbons interacted with the -SH or S-S groups of the proteins in a manner which prevented the detection of these groups by the analytical procedures which we employed. The binding of the hydrocarbons to the proteins, particularly in liver, could conceivably be reflected in the alteration of their metabolic availability to other organs and in the protein content of these organs.

Summary. Phenacyl bromide, p-chlorobromobenzene, or naphthalene tetrachloride inhibit growth of rats which ingested an 8% casein diet. The inhibition was alleviated by supplementary cystine, or by a 27% casein diet. Nitrogen balance remained positive in rats which ingested the 27% casein diet or the 8% casein diet which was supplemented with cystine. Intraperitoneal injection of naphthalene tetrachloride into adult rats on the high or low protein diet induced a negative nitrogen balance, accompanied by a decrease in the -SH and S-S content of the whole blood and in the protein content of practically all tissues. The implication of these observations is discussed in terms of possible interference of the hydrocarbons with the protein metabolism of the rat via direct binding of the hydrocarbons to the proteins through the -SH or S-S groups.

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Coagulation-Promoting and Inhibitory Properties of Modified Thrombin Preparations.‡ (22780)

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The fibrinogen-clotting activity of thrombin is known to be destroyed by heating above 60°C(1). As will be shown here, such heated preparations have profound effects on various phases of the coagulation mechanism.

Materials and methods. Tropical thrombin;* bovine fibrinogen, Fraction I;† human plasma; bovine plasma; human platelet material; degradation products of thrombin. Human plasma was prepared from normal blood which had been treated with a solution of sodium oxalate (1 part of 0.1 M oxalate solution in water to 9 parts of blood) and was centrifuged at 32,000 g for 30 minutes at room temperature. Bovine plasma was prepared in a similar manner. Human platelet material was prepared by the method described previously(2). Inhibitors of thrombin were prepared by heating solutions of thrombin in isotonic saline in the waterbath for varying periods at temperatures ranging from 40°C to 100°C. In most of the work reported here, the material studied was ob-

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† Armour and Co., Chicago, Ill.