creases the dissociation of the phenolic hydroxyl groups(13). The resultant increased acidity of the iodinated molecules may be responsible for the separation from the bulk of non-iodinated molecules on the DEAE-cellulose column.

Radiation may alter albumin. A total dose of 50.000 roentgen equivalent physical (rep) to albumin in a solution of 0.2 mg albumin ml can alter its biologic properties(14). However, increasing the albumin concentration to 5 mg ml partially protects against the damaging effects of irradiation. The RISA-T3 preparation used here was exposed to a relatively low radiation dose, receiving approximately 10.000 rep in a solution containing 50 mg of albumin per ml. Also, the biologic characteristics of this I^{131} albumin preparation compare favorably with those previously reported(2). Following intravenous administration to man of RISA-T3 preparations, a constant rate of iodinated albumin degradation during 30-day periods of observation has been repeatedly observed.† This would suggest that the iodinated albumin preparation that was chromatographed had not been severely damaged by radiation, for radiation damaged albumin preparations contained variable amounts of rapidly degraded iodinated albumins.

Studies to determine which of the many possibilities discussed above were responsible for altering the iodinated albumin so that its chromatographic behavior differed from that of native serum albumin are being undertaken. Further, the significance of these chromatographic differences in terms of biologic properties remains to be elicited.

Summary. The chromatographic behavior of radioiodinated albumin preparations was found to differ markedly from that of native serum albumin on anion-exchange (DEAE-) cellulose adsorbent columns. The significance of this observation is discussed.

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Received October 7, 1957. P.S.E.B.M., 1958, v97.

Effect of p-Dimethylaminoazobenzene and β-Naphthylamine on Rat Liver Sulfhydryl Concentration.* (23717)

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Various experimental procedures and numerous agents will influence the total non-protein sulfhydryl (TNPSH) content of rat and mouse livers(1-8). Scalding, ligation trauma, exposure to cold or shock, restraint and subcutaneous injections of adrenalin, have been shown to cause a marked drop in the total non-protein sulfhydryl concentration. Over the past 2 years(9,10), research conducted in this department has revealed

* Supported by the Elks Cancer Fund through Purdue Research Fn.

that nonnarcotic analgesics such as acetylsalicylic acid, salicylamide and acetophenetidin inhibited the decrease of the total non-protein sulfhydryl content in the liver of the rat subjected to stress. Aminopyrine and sodium salicylate failed to inhibit the decrease of this sulfhydryl content of the stressed rat liver. Early reports(11-15) suggested the interaction of carcinogens with SH-groups of cell proteins and particularly of enzyme proteins, and recent investigations(16-19) pointed out that carcinogenic agents must in some way interfere with cell constituents in order to exert their cytochemical effects. Therefore, a study was made of the influence of carcinogenic agents on the total non-protein sulfhydryl concentration of the rat liver.

The purpose of these studies was 3-fold: (1) To compare the effects of stock and low protein (8%)-low riboflavin diets on the total non-protein sulfhydryl (TNPSH) content of the rat liver; (2) to determine the changes induced in the total non-protein sulfhydryl level by the addition of p-Dimethylaminoazobenzene (DAB), commonly known as "Butter Yellow" and β-Naphthylamine (BNA), designated as 2-Naphthylamine, to these diets; (3) to ascertain whether or not there is any correlation between the liver non-protein sulfhydryl concentration and phases of carcinogenesis under these experimental conditions.

Materials and methods. The method for determining the TNPSH was basically that developed by Kolthoff and Harris(20), as modified by Benesch and Benesch(21,22) and applied by Takesue and Miya(10). The animal is sacrificed by cervical dislocation. The liver is removed and immediately frozen in dry ice. A portion (440-425 mg) is cut and weighed. This tissue is treated with 10 parts of 2.5% sulfosalicylic acid in an all glass tissue grinder and ground quickly. After centrifugation and filtration through shark-skin filter paper, a 1 ml aliquot is placed in the titration mixture consisting of not less than 85% ethanol, with the addition of sufficient ammonium hydroxide and ammonium nitrate to make the concentrations 0.25 M and 0.05 M respectively. The titration mixture is made 3 x 10^{-5} N in ethylenediamine tetraacetate tetrasodium salt. The mixture is titrated at the rotating platinum electrode with 0.0005N silver nitrate solution. The theoretical end point of the titration is obtained graphically by plotting current readings (microamperes) against volume of silver nitrate solution added and noting the intersection of the two straight lines. The results are presented as mg% of TNPSH calculated on the wet weight of the liver. The TNPSH content of the liver of normal fed and fasted (20-24 hours) female Sprague-Dawley rats was determined amperometrically. The values found agree quite well with those reported by Takesue and Miya(10).

TABLE I. Effects of Fasting and Acute Doses of Carcinogenic Agents on the TNPSH Content of the Rat Liver.

No. of animals	Treatment	Avg, mg %*	± S.E.
6	None	302.8	5.2
10	Fasting (F), 20-24 hr; water *ad lib*†	282.7	3.0
4	F; DAB, 10 mg/kg	300.0	5.3
4	20	274.0	9.7
4	50	259.0	12.6
4	F; BNA, 5	272.0	5.9
4	10	201.2	6.2
5	20	208.5	3.0

* Avg of duplicate titrations.
† Used as controls for subsequent work.

Results. Acute experiments. Groups of 4-5 female Sprague-Dawley rats, fasted for 20-24 hours with water *ad libitum* prior to the experiment, received 10 mg, 20 mg, 50 mg, per kg of DAB and 5 mg, 10 mg, 20 mg, per kg of BNA respectively. The carcinogenic agents were suspended in a 0.2% solution of sodium alginate in water and administered orally by means of a stomach tube. All concentrations of the carcinogens were suspended in this agent in such a way that the volume of solution administered to the animals remained constant. Four to 5 hours after the gavage, the rats were sacrificed and the sulfhydryl determination made.

The results, Table I, indicate that fasting alone (20-24 hours) had very little effect on the TNPSH content of the rat liver. In acute experiments, DAB, administered up to 50 mg/kg, failed to produce a marked decrease

in the sulfhydryl level, whereas BNA, given at a dose of either 10 mg or 20 mg kg, induced a significant and similar drop in the TNPSH concentration. A dose of 5 mg/kg of BNA was without effect.

Chronic experiments. The main objective of this problem was to determine the changes induced in the total non-protein sulfhydryl (TNPSH) level by addition of p-Dimethylaminoazobenzene (DAB) and β-Naphthylamine (BNA) to experimental diets. Reports have emphasized the importance of the diet and its riboflavin content during the process of liver cancer(23,24). Consequently, diets having a well-defined composition from a nutritional standpoint, have been used throughout the entire experiment for the purpose of studying primarily the influence of DAB and BNA on the sulfhydryl level of the rat liver.

Female Sprague-Dawley rats, weighing approximately 140 g at the onset of the experiment, were maintained on 2 different diets: a stock Purina Laboratory Chow powder and a low protein (8%) diet† with a low riboflavin content (2 mg/kg diet). In each instance, the carcinogens were incorporated thoroughly in the food, 600 mg of DAB or 100 mg of BNA per 1 kg of diet. To secure uniform distribution of the carcinogens in the diets, mechanical mixing of at least 1 hour was provided by an electrical ribbon mixer. These diets were fed to the rats daily and no measurement of the individual food intake was made. In order to prevent accumulation of old food, the food cups were refilled each day. The average food consumption was good and the weight loss recorded was not due to refusal of rats to eat the experimental diets. For each group of rats fed a diet containing DAB or BNA, control animals were fed the same diets without the carcinogens. The feeding period of the deficient diet was 16 weeks and the normal diet 24 weeks. All animals were weighed weekly and their weight recorded. At the beginning of the experiments the average weight of the animals in all groups was about 140 g; at 16 weeks average weights in the various groups were as follows: Normal diet, 247; low protein diet, 214; low protein and DAB, 167; low protein and BNA, 214, normal diet and DAB, 229; normal diet and BNA, 257. The animals on the low protein diet plus DAB lost weight at the 6th week and gained the least during the experimental period. To determine under such well defined nutritional conditions the effects of the carcinogens on the sulfhydryl content, 5 animals of each control and experimental group were selected at random every 4 weeks and the TNPSH concentration determined as previously described. Food was withheld from the animals 20-24 hours with water *ad libitum* prior to the experiment. At the time of sacrifice, portions of the liver, adrenal, spleen, pancreas, kidney and bladder of each rat were fixed for histopathologic examination.

Table II gives a summary of the liver sulfhydryl level after prolonged treatment. The data, from the chronic experiments, revealed that the TNPSH level of the stock diet fed rats remained relatively constant throughout the experiment. The low protein diet fed rats showed a decrease after 12 weeks but the liver TNPSH values returned to near normal in 16 weeks. There is also indication that the addition of 600 mg of DAB or 100 mg of BNA per 1 kg of diet decreased the TNPSH concentration of the rat liver. The incorporation of BNA to a non-protective diet caused a maximum drop after 4 weeks. Under the same experimental conditions, the effect of DAB was quite marked after 8 weeks with very little variation at the end of 16 weeks.

These carcinogens, when added to the stock diet, likewise decreased the liver sulfhydryl level. In this instance, the results indicate that both DAB and BNA induced their maximum decrease after 12 weeks, with a gradual return to normal values at the 20th week.

Tissue Pathology Studies.‡ Examination of the tissues, at the time of sacrifice, showed a few cases of enlarged spleens and kidneys and no gross changes in the pancreas, bladders, and adrenals of the experimental animals. The livers of the deficient diet-DAB fed rats exhibited more severe gross pathologic

† Nutritional Biochemicals Corp., Cleveland, O.

‡ The authors are indebted to Dr. A. L. Delez, Veterinary Science Dept., for interpretation of the histopathologic sections.

TABLE II. Effects of Carcinogenic Agents on TNPSH Content of Rat Liver.*

Diet	Weeks 4	8	12	16	20	24
Normal, control	281.5	285.0	285.0	281.0	295.0	290.0
	2.9	6.0	3.1	4.0	4.0	7.6
Low protein, control	281.0	271.5	221.0	275.0		
	3.2	10.3	6.7	8.1		
Low protein & DAB†	279.5	198.0	181.0	193.0		
	9.4	12.4	5.7	11.5		
Low protein & BNA‡	181.5	222.5	200.0	230.0		
	14.9	6.0	2.0	6.1		
Normal & DAB†	307.0	293.0	218.5	265.0	314.0	263.0
	5.3	5.3	6.3	17.8	2.4	5.5
Normal & BNA‡	282.0	272.0	225.0	257.0	304.0	282.0
	13.3	14.9	8.0	8.0	6.0	8.4

* Avg mg % (± S.E.). † 600 mg of DAB added to 1 kg of diet. ‡ 100 mg of BNA added to 1 kg of diet.

changes than those of the stock diet-DAB fed rats. The pancreas, spleens, adrenals, kidneys and bladders had no evidence of significant histopathologic changes.

Significant tissue abnormalities occurred in the livers of rats under DAB treatment. A severe degeneration of the parenchymal cells was observed in the low protein-diet fed rats. This lesion is associated with the appearance of abnormal parenchymal cells which have large hyperchromatic nuclei. These features may represent the early stages of neoplastic formation in the organ.

Discussion. The exact mechanism by which these carcinogens exert their effects on the TNPSH content of the rat liver, when added to a diet, is unknown. The results suggest that the low protein diet creates a type of stress which manifests itself in a decrease in the liver TNPSH content and that the carcinogenic agents will augment the severity and the acuteness of the stress condition. It is also obvious that DAB and BNA act independently of the stress state induced by the non-protective diet, causing a sulfhydryl depletion even with the stock diet. The sulfhydryl compounds, as Barron(25) pointed out, are indispensable requirements for processes of growth, enzyme activity and metabolism. It is reasonable to assume that a detoxication of the carcinogens, which takes place in the body, requires and binds sulfhydryl groups, in this manner depriving the organism of a vital factor of normal growth. Whether or not the sulfhydryl derivatives, which we assume to be formed in the body, may be intermediates in either the detoxification mechanism, the process of carcinogenesis, or both remains to be elucidated. The fact that the TNPSH level of the low protein diet control group at 12 weeks was significantly low indicates that the diet might have played an important if not a primary role in TNPSH depletion. Indeed, it has been shown that liver tumor growth is facilitated by such diets.

Summary. An investigation was made of the effects of 2 carcinogenic agents on the total non-protein sulfhydryl content of the rat liver. It was found that fasting decreased the sulfhydryl content only slightly. Acute doses of β-Naphthylamine produced a marked lowering of the total non-protein sulfhydryl concentration, while p-Dimethylaminoazobenzene failed to elicit this effect. It was possible to demonstrate that the total non-protein sulfhydryl level of the stock diet fed rats remained relatively constant throughout the experiment and that low protein-low riboflavin diet created a type of stress which manifested itself in a decrease in the total non-protein sulfhydryl compounds of the rat liver. This study indicates that addition of 600 mg of p-Dimethylaminoazobenzene or 100 mg of β-Naphthylamine/kg of diet induced a decrease in the total non-protein sulfhydryl content of the liver. The sulfhydryl depletion, which takes place under such experimental conditions, may be involved in either the detoxica-

tion mechanism, the process of carcinogenesis, or both. However, it is to be emphasized that the data do not permit any conclusion with respect to the relationship between carcinogenesis and liver sulfhydryl concentration.

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Received October 18, 1957. P.S.E.B.M., 1958, v97.

Acute Choline Deficiency in Albino Rats: Vascular Contractility, Vascular Fragility, and Blood Pressure.* (23718)

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The rapidly developing and spectacular effects of choline deficiency in the weanling albino rat have been described(1-6). The most conspicuous feature of the deficiency syndrome is hemorrhagic degeneration of the kidneys, although hemorrhages are also observed in many other organs including eyes, heart, adrenals, liver and brain. The present study is concerned with an attempt to quantitate changes in vascular contractility and fragility during the syndrome, by technics to be described, and includes observations of blood pressure made during the course of the investigation.

* This study was supported in part by Nutrition Fn., N. Y., and the A. H. Robins Co., Richmond, Va.

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Methods. Composition of deficient diet was: casein 18%; lard 30%; cornstarch 30%; sugar 12%; agar 2%; salt mixture 4%; cod liver oil 4%; wheat germ oil 1%; salad oil 1%; and thiamine-8 mg, riboflavin-8 mg, pyridoxine-8 mg, calcium pantothenate-15 mg, nicotinic acid-15 mg, nicotinamide-15 mg/kilo of diet. A preliminary trial demonstrated that this diet produced typical findings of acute choline deficiency in albino weanlings, with mortality of 25, 62, or 40%, within 12 days. The supplemented diet was prepared by adding to the test diet choline chloride 500 mg/k, methionine 1 g/k, and inositol 100 mg/k. Twenty-five weanlings receiving this latter diet for 3 weeks showed a normal growth curve, and autopsies revealed no evidence of choline (or other) deficiency. 116 male weanling albino rats of Wistar