Effect of Fresh Milk on the Production of Hepatic Tumors in Rats by Dimethylaminoazobenzene

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The prevalence of primary cancer of the liver in Africa and in certain parts of Asia is a problem that should attract increasing attention. In a series of papers Berman (1, 2, 3) describes its frequency and pathology amongst the Bantu in South Africa, and in discussing the possible etiological factors (4) draws attention to its coincidence with cirrhosis. He enumerates a number of other conditions (helminthiasis, malaria, schistosomiasis, hemochromatosis, alcohol) that may perhaps be responsible for those changes in the liver to which some races seem more susceptible than others. None of these factors, however, is present in all the communities where primary liver cancer is prevalent.

The question of racial susceptibility was investigated by Kennaway (16), who analyzed the data concerning the incidence of primary liver cancer amongst Negroes in America and Africa; for since the former are descendants of the West African Negroes they might have the same racial susceptibilities. But the scanty data available did not indicate that the incidence of primary liver cancer amongst American Negroes differs from that among the white people in America, and if this is so the very high incidence in African Negroes cannot be of purely racial character. In Africa some extrinsic factor may be involved.

This extrinsic factor could be related to one of the agents described by Berman as provoking cirrhosis of the liver, but none of these was found to be consistently connected with the occurrence of liver tumors. A second possibility is that materials are present in the food of peoples liable to liver cancer that may evoke the development of these tumors; or substances are absent that may prevent the formation of such neoplasms.

Investigations concerning the first possibility are in progress. With regard to the second, experiments with hepatic tumors induced by *p*-dimethylaminoazobenzene seemed suitable, as it has been shown that these growths are affected by modifications in the diet. Yeast and liver (19-21), both of which are rich in vitamins of the B complex, and chemically pure substances, riboflavin and casein (13, 17), separately or in combination, were found effective in preventing tumor formation. It seems that the quantitative relations of these substances to other vitamins, *e.g.*, biotin, which is "procarcinogenic," (7, 27), play a determining role (8). It has, however, been questioned whether the prevention of neoplasms by yeast is connected with its vitamin content, and it was suggested that *p*-dimethylaminoazobenzene becomes adsorbed on yeast residues and so escapes resorption from the alimentary canal (14). Hydrogenated cocoanut oil also was found effective in preventing the formation of liver tumors by *p*-dimethylaminoazobenzene (18).

With regard to dietary factors that may influence the development of hepatic neoplasms in human subjects, it was of interest to investigate widely used foodstuffs, and the present experiments are concerned with the effect of fresh milk on the production of tumors in the liver of rats.

EXPERIMENTAL

All rats used were albinos of approximately 100 gm. body weight at the beginning of the experiments. The basis of the diet in this series of experiments was polished rice; the supplements and the caloric values of the main components are given in Table I.

Diet

p-Dimethylaminoazobenzene was added as a 3 per cent solution in arachis oil; 2 ml. of the solution to 100 gm. of rice gives a concentration of 0.06 per cent of *p*-dimethylaminoazobenzene. Azobenzene was fed in the same way. In controls 2 ml. of arachis oil was added to 100 gm. of rice, or no oil was added. Fat-free and vitamin-free casein in amounts of 6 gm. added to 100 gm. of rice was used. The amount of casein was so chosen as to give about the same amount of protein as is contained in the diet supplemented with milk. The food mixture was freshly prepared every day and given in measured quantities such that all had usually been consumed by the next day. The animals were permitted free access to it, and any food remnants were weighed and subtracted from the total food given. Animals on the milk diet received amounts equivalent to 10 ml. of fresh milk each daily, but the amount drunk by each individual was not known. Cabbage was given once weekly to all rats and to those not receiving milk carrots were fed in amounts of 1 to 2 gm. a day

TABLE I: SUPPLEMENTS ADDED DAILY TO POLISHED RICE BASAL DIET

Experiment started	Addition	Sex of animals	No. of animals	Caloric value of the diet*: Total Calories in 100 gm. food	Percentage caloric value in diet of: Carbohydrate	Protein	Fat
July 12, 1944	Carrots + 2% arachis oil	♂	10				
	" " "	♀	10	412	86.9	6.8	6.3
	" " " + 0.06% *p*-DA	♂	10				
	" " " " "	♀	20				
Nov. 23, 1944	6% Casein + 2% arachis oil + carrots	♀	10				
	6% Casein + 2% arachis oil + carrots + 0.06% *p*-DA	♀	30	436	82	12	6
Dec. 1, 1943	10 ml. Milk + 2% arachis oil	♂	30				
	" " " "	♀	20				
	" " " " + 0.06% *p*-DA	♂	30	477	78.6	8.7	12.7
	" " " " " "	♀	20				
July 3, 1945	" " " " " "	♂	10				
	" " " " " "	♀	20				
Jan. 20, 1944	10 ml. Milk only	♂	20	460	81.6	8.9	9.5
		♀	10				
Oct. 20, 1944	Carrots	♀	10	395	90.5	7.1	2.7
Oct. 20, 1944	Carrots + 2% arachis oil + 0.06% azobenzene	♀	10	412	86.9	6.8	6.3

* Calculated from values given by McCance and Widdowson: "The Chemical Composition of Food" (1940).
p-DA = *p*-Dimethylaminoazobenzene.

FOOD INTAKE AND BODY WEIGHT

The weights of the animals were recorded weekly. Rats were killed at intervals of 3 to 7 days at the beginning of the experiment, later at longer intervals, and the organs weighed immediately and fixed in formalin.

RESULTS

Figs. 1 to 5 show the food intake and body weight of the rats during the experiment. Males and females are recorded separately. The males ate about 30 per cent more than the females.

(a) Where milk was given, the food intake of males

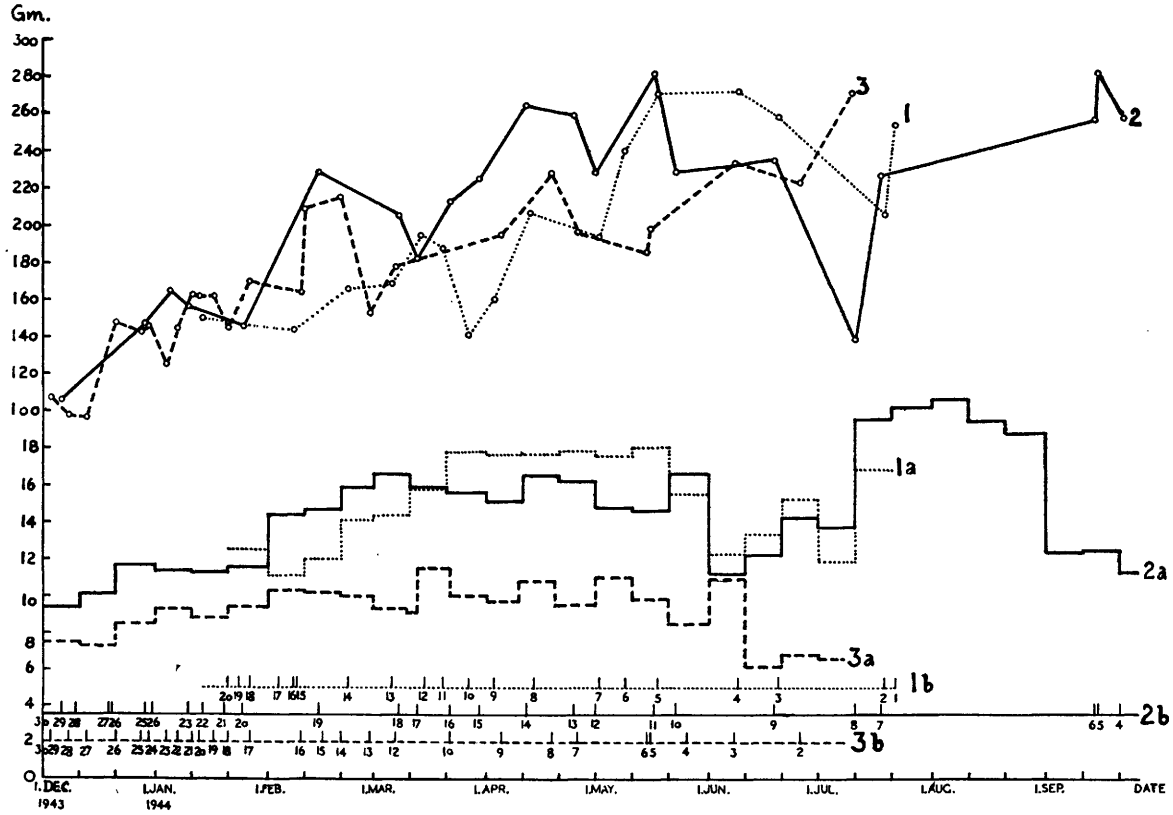

FIG. 1.—Growth and food intake of male rats on various diets. Upper three curves (1, 2, and 3) represent body weights of individual animals on day when they were killed; lower three (1a, 2a, 3a) represent average food intake during 10 day periods per animal per day. Numbers below straight lines (1b, 2b, 3b) represent number of rats surviving in corresponding groups of 1a, 2a, and 3a.

. . . . . = Rats receiving rice and milk. ——— = Rats receiving rice, 2 per cent arachis oil, and milk. - - - - - - = Rats receiving rice, 2 per cent arachis oil, 0.06 per cent *p*-dimethylaminoazobenzene, and milk.

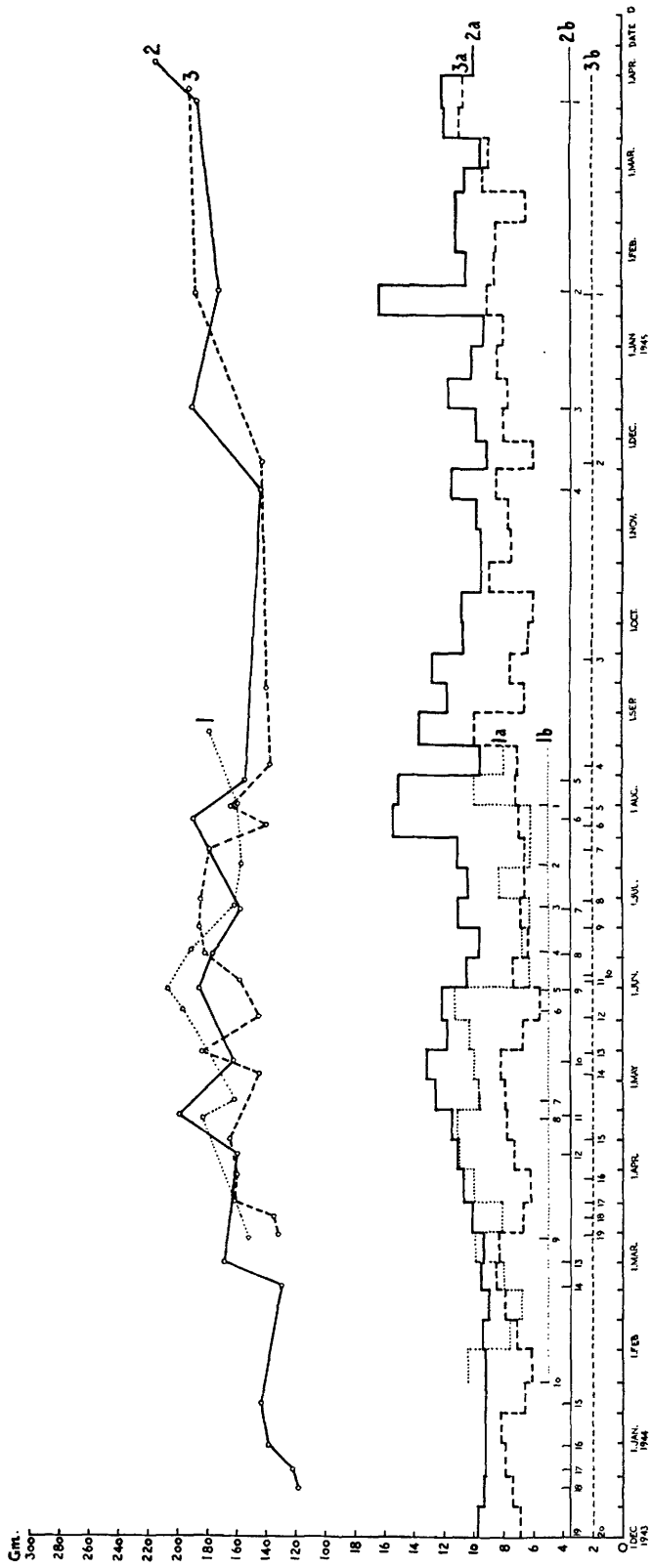

FIG. 2.—Growth and food intake of female rats on various diets. Upper three curves (1, 2, and 3) represent the body weights of individual animals on day when they were killed; lower three (1a, 2a, 3a) represent average food intake during 10 day periods per animal per day. Numbers below straight lines (1b, 2b, 3b) represent number of rats surviving in corresponding groups of 1a, 2a, and 3a.

. . . . . = Rats receiving rice and milk. ——— = Rats receiving rice, 2 per cent arachis oil, and milk. - - - - - - = Rats receiving rice, 2 per cent arachis oil, 0.06 per cent *p*-dimethylaminoazobenzene, and milk.

not receiving *p*-dimethylaminoazobenzene was about 40 per cent and that of the females about 50 per cent higher than that of animals on the same regimen but receiving *p*-dimethylaminoazobenzene. In spite of the difference in the food intake only small variations could be noted between the body weight of the males on this diet with and without *p*-dimethylaminoazobenzene. No difference was observed between the two

It is remarkable that on the milk diet, which permitted full growth of the rats, the addition of *p*-dimethylaminoazobenzene affected the food intake but not the growth. In the case of diets by which the growth of the body was checked the addition of *p*-dimethylaminoazobenzene did not further reduce the food intake. The body weights of these animals declined during tumor development.

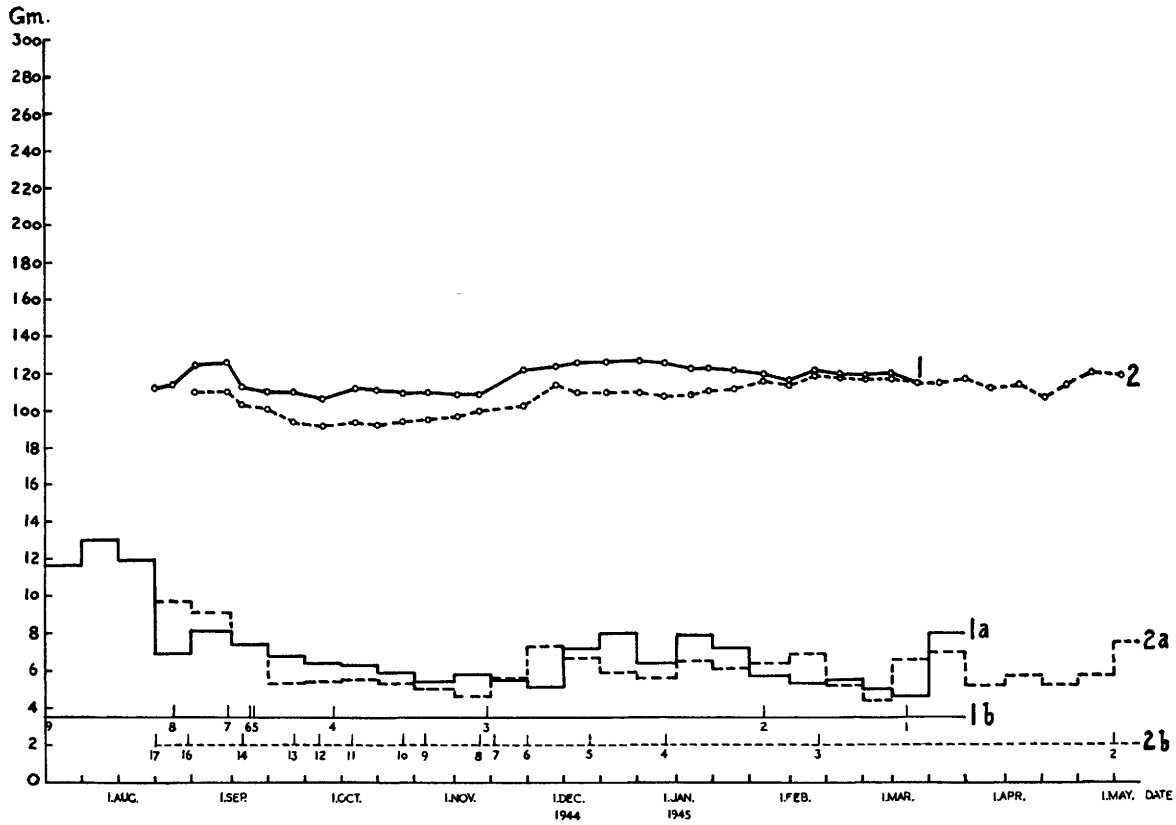

FIG. 3.—Growth and food intake of female rats on diet of rice, carrots, and 2 per cent arachis oil with and without addition of *p*-dimethylaminoazobenzene. Upper two curves (1 and 2) represent means of body weights; lower two (1a, 2a) represent average food intake during 10 day periods per animal per day. Numbers below straight lines (1b, 2b) represent number of rats surviving in corresponding groups.

———— = Rats on basic diet. - - - - - - = Rats receiving addition of *p*-dimethylaminoazobenzene.

corresponding groups of females. The weights of the rats receiving milk increased about 1.5 gm. daily and reached about 270 gm. in the males and about 220 gm. in the females.

(b) The food intake of the animals on the diet of polished rice with or without the addition of casein fell to 40 per cent of that of the animals receiving milk. No greater reduction in the food intake took place with animals not receiving milk when *p*-dimethylaminoazobenzene was given. The growth of the rats was retarded or completely stopped, whether the diet contained *p*-dimethylaminoazobenzene or not; in many cases animals receiving *p*-dimethylaminoazobenzene lost weight.

CHANGES IN THE LIVER

Fig. 6 shows the incidence and time of occurrence of hepatic tumors in rats on the different diets. The liver weight of all animals receiving *p*-dimethylaminoazobenzene was greater than that of the controls without *p*-dimethylaminoazobenzene, and enlargement of this organ was greater in the groups on diets not supplemented with milk (Table II). The increase of the liver weight was not due to an increased water content, as the percentage dry weight of all livers was approximately the same.

Histological investigation of the livers of animals on all diets without *p*-dimethylaminoazobenzene

showed no abnormalities, apart from occasional slight fatty changes.

1. In two series of experiments the diets that contained *p*-dimethylaminoazobenzene were supplemented with fresh milk. In the first series of 50 animals normal livers were found up to 400 days. In 2 livers (from rats killed on the 409th and 465th days) very small patches of early cystic cholangioma were found. Some of these livers showed considerable fatty changes; of the caudate lobe. The growths were multifocal cystic and noncystic cholangiomas, with incipient hepatomas in 2 cases. None metastasized till the 280th day. All the livers showed nodular cirrhosis and/or cholangiofibrosis, but all contained normal regions; usually the right median and right lateral lobes. The only liver that did not bear a tumor, from an animal killed on the 129th day of the experiment, did not show cirrhosis.

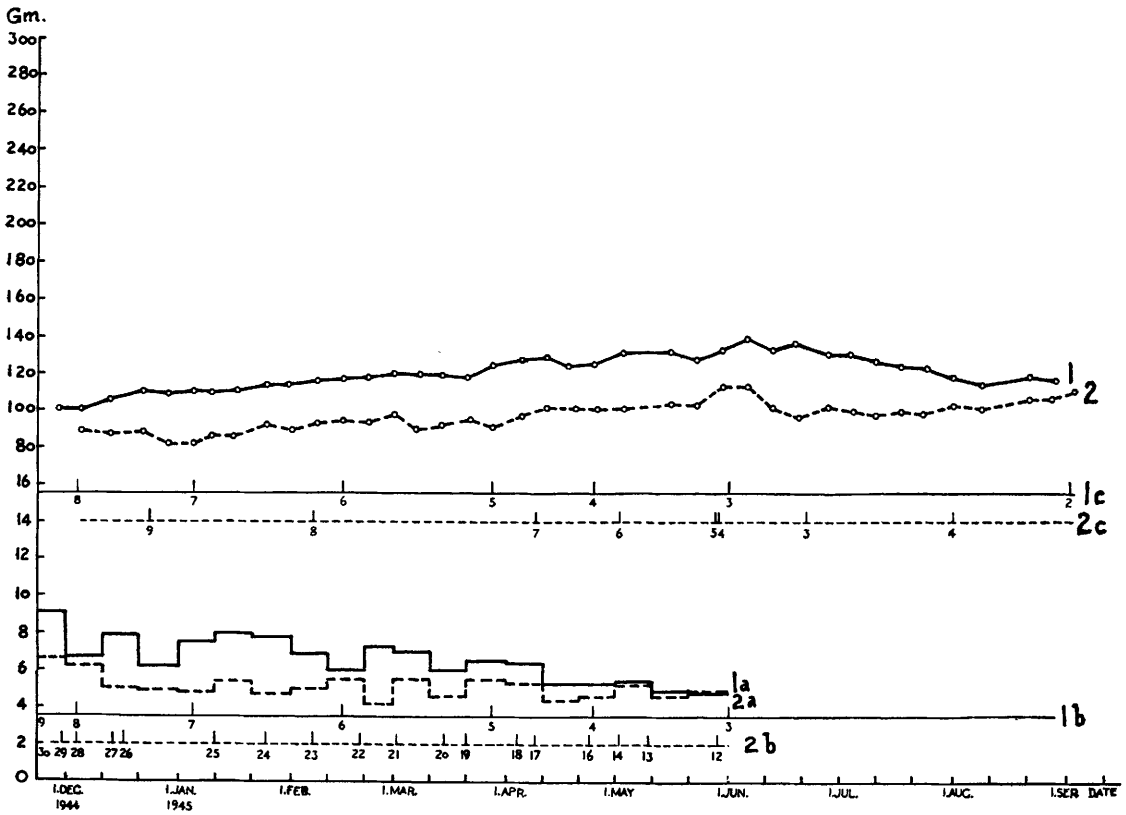

FIG. 4—Growth and food intake of rats on diet of rice, 6 per cent casein, carrots, and 2 per cent arachis oil with and without addition of *p*-dimethylaminoazobenzene. Upper two curves (1 and 2) represent means of body weights; lower two (1a, 2a) represent average food intake during 10 day periods per animal per day. Numbers along straight lines below each pair of curves represent number of rats surviving in corresponding groups; 1c and 2c refer to 1 and 2; 1b and 2b, to 1a and 2a.
———— = Rats on basic diet. - - - - - - = Rats receiving addition of *p*-dimethylaminoazobenzene.

none had cirrhosis. In the second series of 30 animals 1 tumor was found on the 161st day, a second on the 262nd day, and two more on the 378th and 386th days respectively. All the tumors were cholangiomas.

2. Of the livers from rats on diets of *p*-dimethylaminoazobenzene and rice one, obtained on the 61st day, showed fatty degeneration and diffuse cirrhosis with an early cholangioma. Tumors were found in the livers of animals killed on the 73rd and 87th days and subsequently in all animals, with one exception, killed after the 90th day. All the tumors were localized in the left middle lobe or in the adjoining part

3. In the group of rats where 6 per cent casein was added to the diet of rice and *p*-dimethylaminoazobenzene the first changes were more or less severe fatty degeneration. An increase in the connective tissues was observed on the 88th day, and cirrhosis and cholangioma on the 100th. The neoplasms in this group seemed to grow more rapidly than those in the group on pure rice diet, and a tumor of 7.5 gm. was found on the 136th day; the number of hepatomas was greater (6) but again, in this group, no metastases were found. Two animals had cholangiomas without cirrhosis. The distribution of fatty degeneration,

nodular cirrhosis, cholangiofibrosis, cholangioma, and hepatoma in animals killed after 70 days appear in Table IV.

4. In the small control series with azobenzene neither hepatic tumors nor cirrhosis was observed.

Effects of *p*-Dimethylaminoazobenzene on the Weights of Other Organs

Spleen, kidney, adrenal, thymus, pituitary, and gonads were weighed in every animal immediately after it was killed. The weights of the organs were calculated as a percentage of the body weight, as well as relative to the kidney weight. The latter comparison was made because comparing the weights of the organs as percentages of the body weights might give too high figures if, for example, the body weights had declined from loss of subcutaneous fat, whereas the weight of the kidney does not change much during a loss of body weight. But since the two calculations yielded very similar relationships only one set of figures, those related to body weight, are given in the table.

The most striking change was the increased splenic weight in all groups receiving azo compounds; in the groups of females it was greatest and most constant in the rats injected with azobenzene. All groups given *p*-dimethylaminoazobenzene in the food had increased splenic weights as compared with the group of control animals, but animals developing tumors in the liver had smaller spleens than the mean of those in the same group without tumors (Table III), save for two exceptions. Apparently the size of the spleen in animals receiving azo compounds is influenced by at least two factors: (a) the compounds effect an increase in size that is not connected with their carcinogenic activity, since azobenzene, which does not elicit tumors in rats, caused the most constant increase in the splenic size; (b) the compounds lessen the splenic weight, a process that is seemingly connected with the development of neoplasms. In an earlier investigation mice in which sarcomas and epitheliomas were produced by the injection of methylcholanthrene were found to have significantly smaller spleens than the control mice (15). Changes in the weights of the other organs attributable

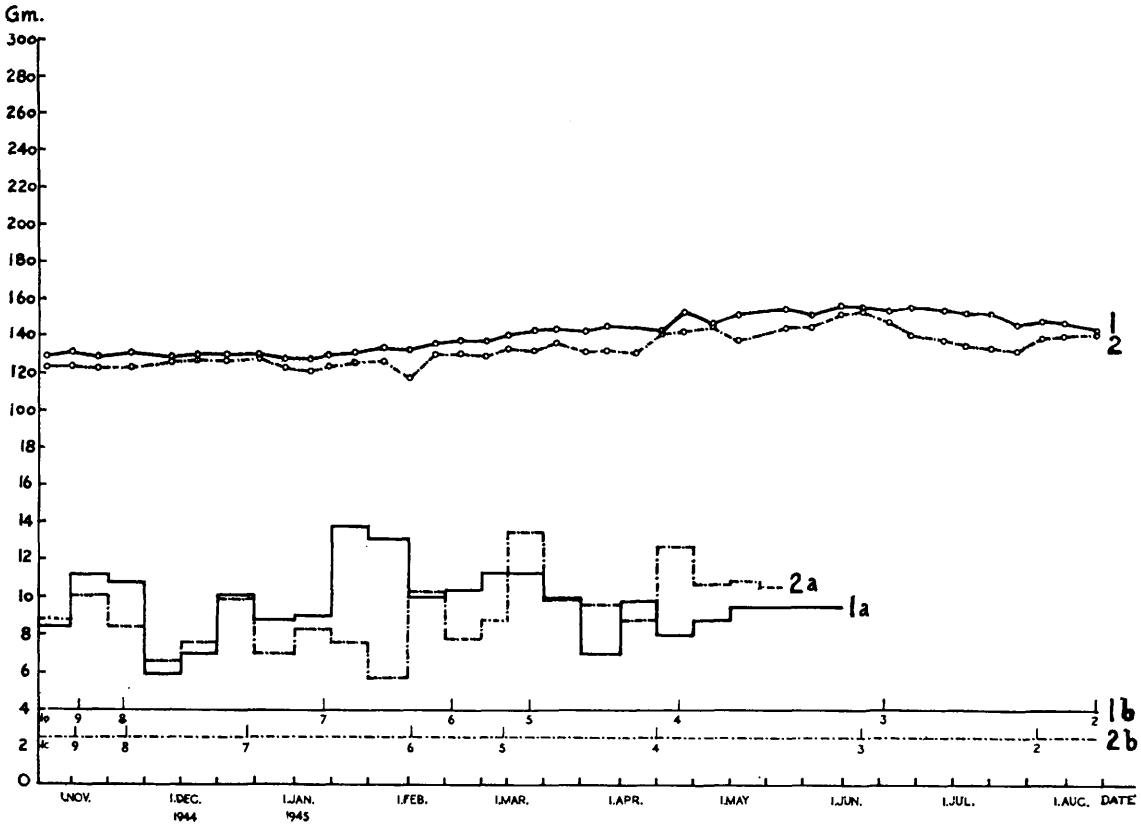

Fig. 5.—Growth and food intake of female rats on diet of rice and carrots with or without addition of azobenzene in arachis oil. Upper two curves (1 and 2) represent means of body weights; lower two (1a, 1b) represent average food intake during 10 day periods per animal per day. Numbers below straight lines (1b, 2b) represent number of rats surviving in corresponding groups. ——— = Animals on basic diet. -·-·-·-·- = Animals receiving addition of azobenzene in arachis oil.

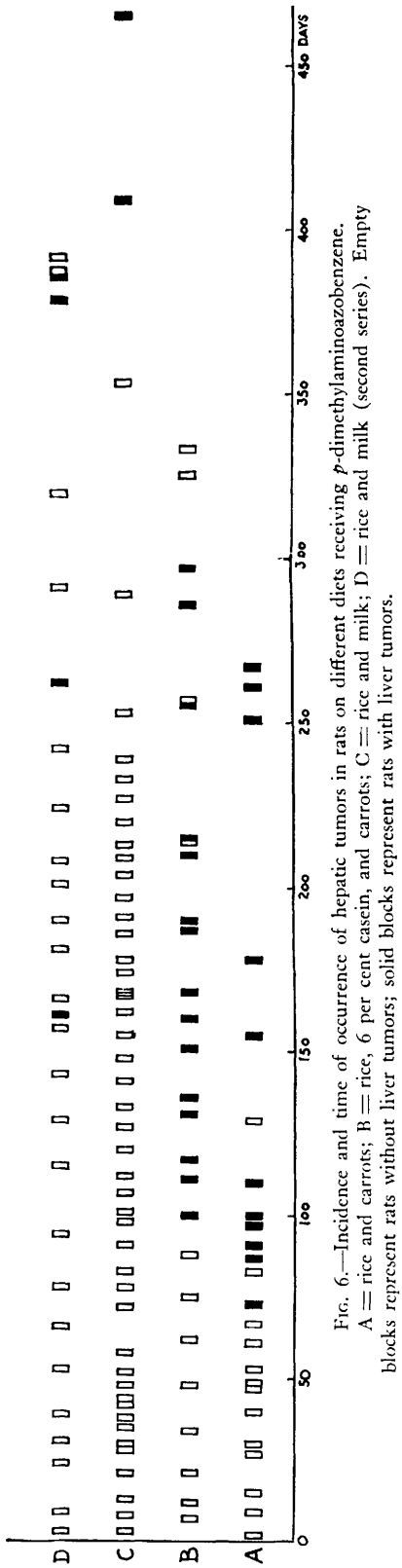

FIG. 6.—Incidence and time of occurrence of hepatic tumors in rats on different diets receiving *p*-dimethylaminoazobenzene. A = rice and carrots; B = rice, 6 per cent casein, and carrots; C = rice and milk; D = rice and milk (second series). Empty blocks represent rats without liver tumors; solid blocks represent rats with liver tumors.

to the carcinogenic dye were not conspicuous, except for those of the ovaries; these are treated in a separate paper. In all groups the adrenals of the males were smaller than those of the females.

CHANGES IN THE STOMACH

By opening the stomachs of animals receiving *p*-dimethylaminoazobenzene it was observed that in some the azo compound was red, and in others yellow. As the color change of *p*-dimethylaminoazobenzene takes place at pH 2.8 to 4.6, no appreciable amount of HCl could have been present when the dye was yellow, and with Congo red as an indicator it was found that in many cases animals receiving *p*-dimethylaminoazobenzene had, in fact, no free HCl in the stomach. On the other hand, nearly all control animals, as well as the animals given *p*-dimethylaminoazobenzene supplemented with milk, had a normal HCl secretion. The stomach content was found acid in all animals when litmus was used as an indicator.

On the 218th day in one animal on a diet of rice, 6 per cent casein, and *p*-dimethylaminoazobenzene, extensive papillomatosis of the forestomach was found, and subsequently this condition was encountered here in 6 out of 6 animals on *p*-dimethylaminoazobenzene. But it was found also in 2 out of 3 rats receiving azobenzene, and in 2 out of 5 control rats.

The diameter of the papillomas ranged from 0.2 to 5 mm.; they were not ulcerated. Microscopically, extensive hyperkeratosis and benign hyperplasia of the squamous epithelium were found. Only in one case, an animal on casein-rice diet with *p*-dimethylaminoazobenzene, was there any sign of downgrowth and penetration of the muscularis mucosae.

DISCUSSION

In the experiments presented here the addition of fresh milk to the diet lessened considerably the production of liver tumors by *p*-dimethylaminoazobenzene in rats fed on rice. While these experiments were in progress a paper by Sugiura (26) appeared, in which it was stated that when 20 per cent of dried milk was added to the diet the incidence of hepatic tumors in rats killed between 132 and 227 days was reduced by 50 per cent; with the addition of 6 per cent dried milk he found tumors in 90 per cent of the animals killed between 150 and 166 days. The rats in the experiments here presented received an amount of fresh milk which would, in the dried state, have made up about 10 to 12 per cent of the whole food taken. This amount, given as fresh milk, protected 80 to 95 per cent of the rats in 2 series of experiments extending over 460 days; thus fresh milk seems to be superior to dried milk in protective activity. It may be that the

TABLE II: WEIGHT OF ORGANS

Supplement to rice diet	No. of animals	Sex	Body		Liver, per cent of body weight		% Dry weight of liver		Spleen, per cent of body weight		Adrenals, mgm. per cent of body weight	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
p-DA + milk	26	♂	270	173	6.34	4.61	33.5	28.86	1.39	0.86	39.1	25.9
			97		3.25		22.8		0.44		14.7	
"	20	♀	192	161	5.68	4.15	32.9	29.09	1.23	0.86	48.4	31.6
			132		3.39		26.7		0.52		22.1	
" "	7	♂	256	180	6.59	4.43	30.2	28.37	1.53	0.90	48.0	33.1
			104		3.35		27.4		0.54		22.6	
" "	14	♀	189	153	4.96	3.82	31.5	29.45	1.11	0.70	72.8	38.2
			110		3.05		28.4		0.46		19.1	
" + carrots + 6% casein	25	♀	133	90	8.0	4.95	32.3	29.65	1.77	1.16	59.0	36.9
			62		3.77		26.8		0.51		23.0	
" + carrots	5	♂	115	104	6.48	5.50	30.0	28.86	1.55	1.45	39.6	33.4
			93		4.70		27.5		1.20		26.2	
" "	17	♀	130	91	6.27	4.76	32.2	28.90	1.50	0.96	56.4	35.6
			57		3.30		25.0		0.63		24.8	
Azobenzene + carrots	9	♀	170	127	5.49	4.41	32.7	30.90	1.60	1.21	80.6	44.3
			114		3.78		29.2		0.64		28.4	
Milk	23	♂	287	214	5.25	3.52	38.0	29.51	0.92	0.45	46.4	25.0
			106		2.56		25.8		0.28		9.2	
"	19	♀	215	165	5.42	3.55	33.5	29.93	1.02	0.51	52.9	38.7
			123		2.59		26.4		0.33		27.0	
" *	16	♂	271	205	5.73	3.79	31.2	29.20	0.72	0.54	33.1	22.8
			141		2.84		28.0		0.30		14.5	
" *	10	♀	207	175	3.80	3.32	36.0	30.47	0.67	0.45	38.5	29.3
			152		2.89		25.0		0.32		10.0	
Casein + carrots	9	♀	134	110	4.53	3.61	33.2	29.63	0.90	0.47	55.5	39.1
			95		2.98		29.3		0.33		30.6	
Carrots	7	♂	132	110	6.61	5.23	34.0	30.03	1.01	0.67	57.6	33.3
			67		3.97		27.0		0.43		10.6	
"	8	♀	129	108	5.01	4.08	30.0	28.86	0.67	0.51	49.4	35.1
			81		3.50		27.5		0.35		24.1	
" *	10	♀	175	127	3.62	3.41	32.8	29.80	0.60	0.43	47.9	35.5
			94		3.18		27.9		0.30		25.0	

* All diets contain 2% arachis oil except those marked with asterisk.

TABLE III: WEIGHTS OF THE SPLEENS OF RATS RECEIVING *p*-DIMETHYLAMINOAZOBENZENE WITHOUT OR WITH TUMORS IN THE LIVER

Diet	No tumors	Tumor in liver.
Rice + *p*-DA	1.76	1.13
	1.62	1.00
	1.54	0.85
	1.50	0.82
	1.45	0.78
	1.12	0.66
	1.01	0.38
	0.94	0.38
	0.84	
	0.83	
	0.81	
	0.74	
	0.73	
	Mean 1.14	Mean 0.75
Rice + 6% casein + *p*-DA	1.50	1.42
	1.42	1.39
	1.38	0.99
	1.28	0.96
	1.11	0.95
	0.93	0.89
	0.93	0.85
	0.89	0.82
	0.72	0.73
	0.56	0.72
	0.43	0.69
		0.63
		0.53
		0.42
	Mean 1.01	Mean 0.85

factors, or some of the factors, involved in the prevention of tumors of the liver are damaged by drying, which may be different according to the process employed. H. Burrows investigated the effect of dried whole milk on the occurrence of mammary cancer in pure (C3H) strain mice, but saw no effect therefrom (private communication).

With full recognition of the danger of accepting results in rats as valid also for the human subject, attention is drawn to a possible analogy in the coincidence of dietary deficiency and the incidence of hepatic cancer among African Negroes. It is extremely difficult to collect data on the nutrition of primitive peoples; in particular it is impossible to give a general answer to the question whether any large proportion of them consume milk. Yet it is fairly certain that in regions of Africa where the tsetse fly is endemic no cow can live, and that the natives have no milk except possibly that from goats. This is the case on the Gold Coast, where nearly all cancer in Negroes originates in the liver (private communication from Dr. P. Goldscheider). In the areas where milk is available its consumption may be restricted because of economic causes or religious customs. In South Africa, in the region of Witwatersrand, milk is available but

TABLE IV

Diet	Duration of treatment, Range in days	No. of animals	Fatty change	Nodular cirrhosis	Cholangio-fibrosis	Chol-angioma	Hepatoma
Rice + *p*-DA	73–267	13	6 (46%)	5 (39%)	4 (39%)	11 (85%)	2 (15%)
Rice + casein + *p*-DA	100–333	19	9 (46%)	9 (46%)	10 (53%)	15 (79%)	6 (32%)
Rice + milk + *p*-DA	72–465	34	8 (24%)	0	1 (3%)	2 (6%)	0
Rice + milk + *p*-DA	78–388	22	7 (32%)	0	3 (14%)	3 (14%)	1 (4.5%)

in consequence of economic restrictions (24) a large part of the population lives very largely on corn. According to Gilbert and his associates (11) the food of natives consist of corn, and "some of the more fortunate Africans are able to supplement their mealie meal[1] diet at regular intervals with 'maas' or fermented milk." By a diet of corn and sour milk, Gillmann and his group (12) were able to produce liver damage, without tumors, in growing rats. We do not know how the milk is altered in this process of souring or fermenting, and whether these milks would still have a protective effect in respect to cancer of the liver. It is stated that in some Bantu communities the women drink milk, but that it is avoided by males. Bourne (5), in his recent survey of the nutrition of the Japanese, points out that the consumption of milk, which was introduced to Japan only at about the end of the 18th century, is negligible. Thus it is clear that one cannot ascertain whether milk is used, even when it is available, without exact knowledge of the local customs, which can be acquired only by workers on the spot.

The data collected by Berman (2) indicate that primary cancer of the liver is prevalent in varying degree over the southern half of Africa, India, the East Indies, China, and Japan. The published material yields the following figures for primary carcinoma of the liver as a percentage of carcinoma of all organs.

	Per cent		Per cent
Bantu	37	Philippines	22
Javanese	36	Semi-Bantu	18.7
Chinese	36	Japanese	7.8
Indians	28		

Over this vast area there is of course an immense variety of dietetic conditions and habits, about which it is difficult to collect complete information and impossible to generalize at present. It seems unlikely that any single positive factor, that is, the same carcinogenic substance in the food, operates over so many different countries, though such a factor may be present in some areas; *e.g.,* Portuguese East Africa (2). A deficiency in the diet is more likely to be common to all these peoples, and this deficiency might be one that is made good by some constituent of milk.

[1] Corn meal.

None of the deficient diets alone produced cirrhosis or hepatic tumors in rats in the present experiment; the presence of a second factor was necessary to induce the formation of neoplasms in the liver. Similarly in cancer of the liver in man some carcinogenic agent may be concerned, the manifestation of which is modified by dietary factors.

As for the relation of neoplasia to cirrhosis and the probable sequence of events leading to the formation of new growths, the results presented here agree with the findings of Opie (22). Cirrhosis is not necessarily a precursor of the tumor, but is frequently associated with it.

The hepatic growths produced in rats by *p*-dimethylaminoazobenzene are multicentric, and cystic and noncystic cholangiomas and hepatomas are found in the same liver, whereas Berman (4) strongly supports the view of the unicentric origin of primary liver tumors in man.

Extensive hyperkeratosis and hyperplasia of the squamous epithelium in the forestomach of rats was first thought to be connected with an infestation by *Gongylonema neoplastica* (10). Passey and his group (23) succeeded in producing similar changes in the stomach of the rat without infestation with *Gongylonema* by using diets adequate in every respect except with regard to vitamin A, and in one series of experiments Cramer (9) found papillomatosis to be produced by vitamin A deficiency, but also by a lack of vitamin B. But some years later, failing to produce papillomatosis by the same means, he concluded that unbalanced diets play only a contributory part and are not the chief factors in the development of papillomatosis. A similar conclusion was reached by Brunschwig and his associates (6), who described extensive hemorrhage and hyperkeratosis in the forestomach of rats as a result of diets unbalanced in protein, fat, or carbohydrate content. The lack of vitamin A, B_1, B_2, or D did not seem to be an etiological factor. Epithelial hyperplasia in the forestomach of the rat on a polished rice diet was observed by Sugiura (25); kieselguhr increased these changes, and they did not occur on unpolished rice. In the present experiment extensive papillomatosis developed in the forestomach of animals on a rice and casein diet, which seemed to be comparable in degree with that of the rats on kieselguhr-rice described by Sugiura. The number of animals with papillomatosis increased on addition

of azobenzene, and was still greater in animals that received *p*-dimethylaminoazobenzene. Their diet was unbalanced in many respects, for they received very little vitamin B and only provitamin A in the form of carotene, mainly from carrots.

The mechanical irritation of the forestomach by hard grains of rice may also have increased the tendency to papillomatosis, while a chemical influence was apparent in the increase of papillomas upon the addition of azo dyes. All animals with papillomas had a reduced gastric HCl.

The addition of fresh milk to the diet prevented the development of papillomas completely and maintained a normal HCl secretion. Milk not only counteracted the effects of an unbalanced diet, but also the noxious effects of the azo dyes. Experiments will be carried out to elucidate the mechanism of these effects.

SUMMARY

1. The addition of 10 ml. of fresh milk daily to a rice-carrot diet containing 0.06 per cent of *p*-dimethylaminoazobenzene protected rats to a considerable extent from the development of liver tumors. In one group of 50 animals 2 incipient growths were found on the 410th and 460th days respectively; in a second group (30 animals) tumors were found on the 161st day, on the 262nd day and on the 378th and 386th days respectively.

2. The addition of 6 per cent of casein to the rice-carrot diet with 0.06 per cent of *p*-dimethylaminoazobenzene did not prevent the formation of tumors, 78 per cent of the animals killed after the 90th day having had hepatic neoplasms.

3. The food intake of the animals decreased on the diet containing milk when *p*-dimethylaminoazobenzene was given. The body weights of the males were slightly lower than those of the controls receiving no *p*-dimethylaminoazobenzene. However, no difference in the weights of females was observed.

4. The food intake of the rats on rice and carrots or rice, casein, and carrots with or without *p*-dimethylaminoazobenzene was about the same, and was about half as great as that of animals on a diet supplemented with milk. The body growth stopped; animals receiving *p*-dimethylaminoazobenzene lost weight slightly.

5. In all groups of rats receiving *p*-dimethylaminoazobenzene the spleen was enlarged. In the same groups the individual rats that developed tumors had smaller spleens than had those without tumors.

6. The free HCl in the stomach generally disappeared in animals on diets producing tumors, but this did not occur in all animals that developed tumors.

7. Some rats on the rice-casein diet developed papillomatosis of the forestomach, the tendency to which was increased by the administration of azo dyes.

8. Possible dietary influences on the development of primary liver cancer in some native peoples of Africa and Asia are discussed.

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