Development of Spontaneous Hepatoma in Mice in Relation to Composition of Dietary Fat.*† (22123)

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The development of spontaneous hepatomas in C_3H mice has been shown to be enhanced by an increased amount of fat in the diet(7,10). Very little, however, is known about the dependence of this tumor on the nature of the dietary fat, of the type demonstrated for the azodye induced hepatoma of the rat(8). In the present study an attempt has been made to find if groups of fat components, derived from the same source, but separated from each other by physical means and fed in equivalent amounts would exert a specific action on the development of the mouse hepatoma.

Methods. Among several methods of fat fractionation(1-5) fractional crystallization from solvents(2,4) presents decisive advantages for nutritional studies. It does not subject fat to chemical reactions, the exact outcome of which it is impossible to predict without precise knowledge of composition of the original fat. It does not involve addition of more than one chemical, which if properly chosen as to its boiling point can be quantitatively removed. Nor does it require heating the fat to higher temperatures, as *e.g.*, molecular distillation. The procedure adopted was as follows: One pound of hydrogenated vegetable fat (H.V.F.)‡ was melted at 50°C, then dissolved in 2 lb of diethyl ether (Purified ether reagent, Merck, glass containers). Ether was chosen as solvent, rather than acetone, because of its lower boiling point. The resulting mixture had a temperature of 35°C. The mixture was cooled for 30-40 minutes under constant mechanical stirring until a temperature of +15°C was reached. The precipitate formed was filtered by gravity at that temperature (Fraction I), filtration requiring about 20 minutes. E. & D. filter paper No. 615, size 33 was used. Precipitate was not washed, but the mother liquor was pressed out as far as possible. The filtrate was cooled further and precipitate formed at +5 (Fraction II), —5 (Fraction III) and −15° (Fraction IV) filtered off similarly. Cooling times required were approximately 40, 50 and 180 minutes, respectively. Filtration time was approximately the same for all fractions. The precipitate obtained at the last temperature constituted "Fraction V." The residues were heated at low pressure until liquefied and remaining ether removed by vacuum distillation at the temperature of liquefaction. This required approximately 4 hours. The filtrate at −15°C was similarly freed of ether at 35°-40°C. Crude fractions thus obtained were allowed to solidify and kept refrigerated until their incorporation into diets. Titer ranges were determined on aliquots of each fraction and are given in Table I. Total yields for each fraction were determined on each lot processed and were found to vary little from one processing to another. Average values are also given in Table I. No attempt has been made to determine exact composition of individual fractions. However, on the basis of extensive analyses of fractions obtained by similar methods reported by other workers it is believed that fractions precipitating at higher temperatures contained a higher proportion of glycerides of long-chain, branched-chain

TABLE I. Melting Ranges and Approximate Recoveries of Hydrogenated Vegetable Fat Fractions.

Fraction	Melting range (°C)	Recovery* (g)
I	55-57	1380
II	44-49	1060
III	37-41	1120
IV	30-33	1640
V	12-26	2000

* Initial amount of material approx. 15000 g.

* Abstract in *Proc. of Am. Assn. of Cancer Research,* 1954, v1, 22.

† This work has been supported in part by grant from Minn. Division of the Amer. Cancer Soc.

‡ H.V.F. = commercial Crisco.

TABLE II. Incidence of Hepatomas.

	H.V.F.	Lard	Fr. I	Fr. II	Fr. III	Fr. IV	Fr. V	P*
No. of tumor bearing mice	8	9	3	0	5	9	13	<.001
No. of mice bearing multiple tumors	1	3	1	0	1	1	3	
Total No. of tumors	9	12	4	0	6	12	20	
Size: <10 mm	4	6	0	0	2	8	12	
>10 mm	5	6	4	0	4	4	8	

* By the χ^2 method.
H.V.F. = Hydrogenated vegetable fat. Fr. = Fraction.

and saturated fatty acids and a smaller proportion of unsaponifiable matter. Composition of diet into which fractions were incorporated was as follows: casein 29%, glucose 34%, yeast 8.2%, Jones Foster salt mixture 4.1%, alfalfa leaf 4.1%, cod liver oil 2.1%, wheat germ oil 1%, neutral fat (lard, hydrogenated vegetable fat or one of the 5 fractions) 17.5%. The diet was made in batches sufficient to last for 3 months at a time. Fractionation was carried out each time a new batch of diet was required. Seven groups of 20 male C_3H mice each were used. Five were fed fractions of hydrogenated vegetable fat while 2 control groups received unfractionated and untreated H.V.F. and lard, respectively. Animals were started on experimental diets at weaning (3 weeks of age, average weight about 10 g). Members of the same litter were distributed among as many groups as possible. Animals were kept 2 to a cage and weighed at weekly intervals. They were fed *ad libitum* from a common food cup, which was left in the cage for one week at a time. At all times they had access to water. All cages were placed in the same part of the room and room temperature did not vary more than 8°F from day to day.

Results. The experiment was terminated after 54 weeks and autopsies performed on all animals. Table II gives the cumulative incidence of hepatomas up to that time. It also summarizes the occurrence of multiple tumors and the breakdown according to size. It can be seen that fractions of low melting point show a markedly greater incidence of hepatomas. Compared with fractions of high melting point there appears to be little corre-

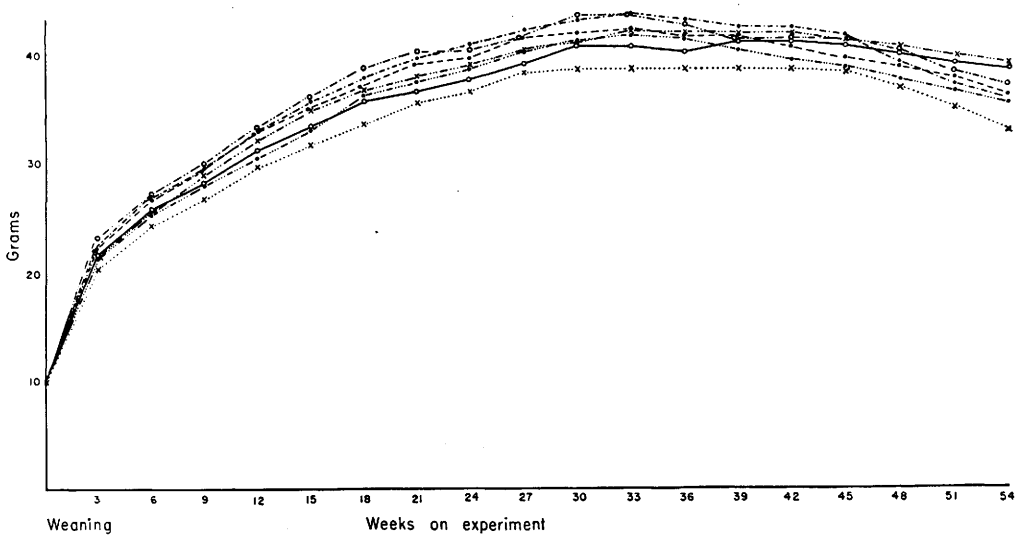

FIG. 1. Weight curves of mice fed different fractions of hydrogenated vegetable fat (H.V.F.). ——— lard, —•••— H.V.F., •••• Fraction I, —•••— Fraction II, —•— Fraction III, ——— Fraction IV, —••— Fraction V.

lation between tumor incidence and tumor size. The occurrence of multiple tumors is sufficiently rare in all groups so as not to permit any conclusions as to the presence of differences.

The foregoing data strongly suggest that the nature of the dietary fat may have a significant bearing on the development of the C_3H mouse hepatoma. The effect observed here is similar to the one reported by Shear and Leiter(9), who fractionated lard by filtration at 38°C and found that the liquid fraction when used as a carrier for benzpyrene gave rise to more tumors than the solid fraction.

It still remains to be established however, whether the differences in the effects are due to qualitative peculiarities of the different fractions or to differences in the actual amounts of fat absorbed. No simple correlation between weight and tumor incidence is apparent from a comparison of the weight curves given in Fig. 1 and the data in Table II. This would suggest a qualitative, rather than a quantitative, difference, but the question as to the nature of the responsible factor remains open for the present.

Summary. Five fractions of hydrogenated vegetable fat (commercial crisco) have been prepared by crystallization from ether at +15, +5, —5, and —15°C. When incorporated in the standard diet at equivalent levels, fractions of low melting point, presumably containing largely unsaturated and shorter-chain fatty acids as well as unsaponifiable matter, are found to give rise to a high proportion of hepatomas in C_3H male mice, while fractions of high melting point give rise to but a few. The magnitude of the observed difference in incidence ($p<.001$) is believed sufficient to justify further studies on the nature of the responsible factors.

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Received October 28, 1955. P.S.E.B.M., 1955, v90.

Collagenase Activity of Dog Pancreatic Juice. (22124)

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Pancreatic juice obtained from pancreatic fistulae artificially created in dogs produces striking digestion of collagen. This is not due to trypsin, chymotrypsin, or carboxypeptidase. It is the purpose of the present work to describe these studies.

Methods and materials. Adult mongrel dogs of both sexes were used. Under aseptic technic the abdomen of the anesthetized animal was opened through a midline incision and the pancreas exposed. The accessory pancreatic duct was dissected free, ligated, and cut, following which the main duct was identified. After placing a ligature close to the point of its entrance into the duodenum, the duct was cannulated with a polyethylene tube whose lumen measured 1.14 mm in diameter; this was tied securely in place. The tube was then brought out through a separate stab incision, following which a Witzel jejun-