TABLE II. Plasma Lipid and Cholesterol Levels of Turkeys Fed Estrogens and Cholesterol.

Treatment	Total lipid* (mg/100 ml)	Total cholesterol* (mg/100 ml)	Total lipid/total cholesterol
0	691 ± 189	184 ± 28	3.76/1
Cholesterol	1,112 ± 359	648 ± 166	1.71/1
DES	13,839 ± 3,095	1,065 ± 266	12.99/1
DES–cholesterol	2,167 ± 660	770 ± 228	2.81/1
DD	16,103 ± 1,390	1,136 ± 261	14.17/1
DD–cholesterol	16,662 ± 3,850	1,967 ± 608	8.46/1

* Mean ± standard error at 11 weeks of age.

The technical assistance of J. W. Carlisle, C. J. Miller and A. Wilder is acknowledged.

1. Simpson, C. F., Harms, R. H., Proc. Soc. Exp. Biol. and Med., 1965, v119, 509.
2. ———, ibid., 1966, v122, 1162.
3. ———, ibid., accepted for publication.
4. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., Circulation, 1952 v6, 858.
5. Prichard R. W., Clarkson, T. B., Lofland, H. B., Arch. Path., 1966, v82, 15.
6. Peck, W. A., Wood, W. D., Raines, M., Dutton, A. M., Oribsen, J. L., Proc. Soc. Exp. Biol. and Med., 1961, v108, 786.
7. Campbell, R. S. F., Lawrie, T. D. V., Pirrie, R., Maclaurin, J. C., Moran, F., Acta Endocrinol., 1960, v34, 207.
8. Simpson, C. F., Harms, R. H., Poultry Sci., 1964, 43, 681.
9. Sperry, W. M., Brand, F. C., J. Biol. Chem., 1955, v213, 69.
10. Pearson, S., Stern, S., McGavack, T. H., J. Clin. Endocrinol. and Metab., 1952, v12, 1245.
11. Pick, R., Stamler, J., Rodbard, S., Katz, L. N., Circulation, 1952, v6, 276.
12. Simpson, C. F., Harms, R. H., Wilson, H. R., Proc. Soc. Exp. Biol. and Med., 1965, v119, 435.
13. Pick, R., Katz, L. N., Johnson, P. J., Century, D. E., Circulation, 1962, v26 667.
14. Lofland, H. B., Clarkson, T. B., Proc. Soc. Exp. Biol. and Med., 1960, v103, 238.
15. Simpson, C. F., Harms, R. H., Exp. Mol. Path., 1966, v5, 183.

Received October 10, 1966. P.S.E.B.M., 1967, v124.

Experimental Studies of Factors Influencing Hepatic Metastases. XVIII. Significance of Trapped Tumor Cells.* (31876)

BERNARD FISHER AND EDWIN R. FISHER
Departments of Surgery and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Partial hepatectomy(1), vena cava constriction(2), reticuloendothelial system blockade(3), mechanical trauma(4), high fat diet (5) and dextran(6) have been observed to increase the incidence as well as size of hepatic metastases following intraportal injection of relatively known numbers of Walker tumor cells in Sprague-Dawley rats. Precise mechanisms responsible for the enhanced metastases have not been elucidated, although evidence suggested that this effect resulted from hepatic damage and/or the trapping of tumor cells within the liver. The present study was performed with ^{51}Cr-labeled tumor cells to determine the significance of increased retention of tumor cells in this regard.

Material and methods. Adult female Sprague-Dawley rats,† weighing approximately 200 g, were employed in all experiments. They were housed in individual cages and, except for those receiving a high fat diet, were permitted Purina Laboratory Chow and tap water *ad libitum*. Animals on the high fat diet were fed a 30% fat, choline-free diet for 4 weeks before tumor cell injection. Their controls were pair-fed with a 3% fat diet during this period.

The Walker carcinoma, propagated in this

* Supported by Am. Cancer Soc. Grant P-142 and USPHS Grants CA-05716, CA-05949 and CA-06670.

† Holtzman Farms, Madison, Wis.

laboratory since 1957, was employed. Details of technics used for preparation, counting, intraportal inoculation and ^{51}Cr-labeling of such cells have been previously described(7,8). ^{51}Cr-labeled cells were suspended in saline or dextran so as to provide a concentration of 500,000 cells per 0.5 ml. The activity of the suspensions used in these experiments averaged 1600 counts/min/0.5 ml.

Animals were sacrificed by exsanguination 1, 4 or 24 hours after tumor cell injection. Livers were removed, washed free of surface blood and blotted dry. ^{51}Cr activity of the median, left lateral and remaining lobes of each liver was separately counted, utilizing a Baird-Atomic sodium iodide thallium activated well scintillation counter.

It was repeatedly demonstrated that a direct relationship existed between the number of tumor cells and radioactivity of all suspensions employed, indicating a random distribution of the chromium tag within the tumor cell population. Results are presented as percent of injected cells or percent of radioactivity.

Technics utilized for partial hepatectomy (1), caval constriction(2) and mechanical trauma (manipulation)(4) of the liver were similar to those previously described in detail.

Reticuloendothelial interference was produced by intravenous injection of shellac-free India ink‡ (32 mg/100 g) 2 hours prior to or immediately after injection of tumor cells. This dose delayed the plasma clearance of Evans blue as noted previously(3).

Two ml of low molecular weight dextran§ (molecular weight 40,000, 10% wt/vol in saline) was injected intravenously 1 hour before tumor cell injection. Tumor cells suspended in low molecular weight dextran instead of saline were also injected intraportally.

Results. Control, untreated rats inoculated intraportally with labeled tumor cells demonstrated 31.1 ± 9.3%, 25.6 ± 7.4% and 23.8 ± 5.1% of the injected activity and/or tumor cells within their livers 1, 4 and 24 hours, respectively, following injection (Table I). The distribution of cells within the hepatic lobes was unequal. One hour following injection, 70% of the liver (left lateral and median lobes) of 17 rats contained 50.1 ± 10.2% of the injected cells, whereas the right lateral and caudate lobes (30% of the liver), which comprise the hepatic remnant following partial hepatectomy, demonstrated 49.9 ± 10.1%.

When partial hepatectomy was performed 24 hours prior to or immediately after tumor cell injection, liver remnants contained the same as or less activity than that contained in a similar portion of liver in control animals. Vena cava constriction, R-E blockade, hepatic manipulation, administration of a high fat diet, and dextran also failed to produce a significantly greater number of tumor cells in the liver (Table I).

Discussion. Previous experiments performed by us(7,8) as well as others(9,10) indicate the usefulness as well as validity of labeling tumor cells with ^{51}Cr. Activity following this procedure is almost exclusively limited to the cellular elements of labeled tumor cell suspensions and such cells are capable of growth into overt tumors when transplanted to suitable hosts. Histologic study has revealed that the administration of such colloidal particles as India ink(3) and dextran(6) results in sinusoidal compression due to swelling of Kupfer cells containing these phagocytized particles. A similar sinusoidal alteration is apparent in the livers of rats receiving a high fat, choline-free ration, in which instance sinusoidal cells are distended with lipid(5). This information as well as recognition of a comparable effect of caval constriction at the site of hepatic outflow on metastases suggest that the enhancing effect of these modalities on metastasis formation within the liver may result from trapping of tumor cells within this organ. A relatively proportional relationship has been noted between numbers of tumor cells inoculated intraportally and incidence and size of hepatic metastases(7). Alteration of the hepatic microcirculation following partial hepatectomy or hepatic manipulation might also be visualized as producing intrahepatic trapping of tumor cells. Yet, the

‡ C 11/1431A, Gunther Wagner, Hannover, Germany.

§ Rheomacrodex, Pharmacia Laboratories, Uppsala, Sweden.

TABLE I. Percent of Intraportally Inoculated ^{51}Cr-Labeled Tumor Cells in Liver.

	Time of sacrifice post tumor cell (TC) injection (hr)		
	1	4	24
I. Controls			
Total liver	31.1 ± 9.3*	25.6 ± 7.4	23.8 ± 5.1
Left lateral and medium lobes (70%)	15.5 ± 4.1	13.4 ± 3.8	13.0 ± 2.2
II. Partial hepatectomy			
A. 24 hr before TC inj	21.3 ± 2.7	12.9 ± 2.8	11.6 ± 2.3
B. Immediately after TC inj	15.6 ± 3.3	8.2 ± 3.0	6.9 ± 1.8
III. Vena cava constriction			
A. Immediately before TC inj	26.9 ± 4.4	28.7 ± 3.7	19.7 ± 3.4
B. Immediately after TC inj	25.1 ± 3.5	25.6 ± 2.6	19.6 ± 2.8
IV. R-E blockade			
A. 2 hr before TC inj	22.9 ± 6.0	22.6 ± 5.3	14.3 ± 4.4
B. 1 hr after TC inj	—	19.2 ± 4.4	11.1 ± 2.2
V. Liver trauma			
A. 15 min before TC inj	27.4 ± 3.2	15.5 ± 2.2	19.6 ± 7.0
B. 15 min after TC inj	30.0 ± 8.1	23.5 ± 10.8	22.0 ± 9.3
VI. High fat diet	30.8 ± 5.4	31.3 ± 5.5	26.2 ± 4.5
VII. Dextran			
A. 1 hr before TC inj	21.3 ± 2.7	12.9 ± 2.8	11.6 ± 2.3
B. TC suspended in dextran	15.6 ± 3.3	8.2 ± 3.1	6.9 ± 1.8

* Each value is the mean and standard deviation of 12 animals.

results of this study fail to substantiate such a contention. Also, investigation of rheologic properties of the blood following hepatic manipulation failed to disclose an increase in blood viscosity(6).

All of the modalities utilized to augment metastases, except dextran infusion, have also been noted to produce elevation of serum glutamic oxalacetic and pyruvic transaminases(1-5), which in the absence of histologic evidence of damage in other tissues signifies hepatocellular damage. Attempts to more precisely define this latter have been unsuccessful. Nevertheless, it appears more plausible to relate the augmentation of hepatic metastases produced by these modalities to the metabolic or biologic ("soil") influences of damaged hepatic cells on tumor growth rather than mechanical factors such as increased trapping of tumor cells.

Summary. No increase in numbers of intraportally injected ^{51}Cr-labeled Walker tumor cells was detected in livers of rats subjected to partial hepatectomy, hepatic manipulation, reticuloendothelial blockade, high fat, choline-free diet, caval constriction, or injection of dextran. This information indicates that the augmentation of hepatic metastases produced by these modalities in this model system is related to their metabolic or biologic ("soil") rather than mechanical influences on hepatic-tumor cell relationships.

1. Fisher, B., Fisher, E. R., Cancer, 1959, v12, 929.
2. ——, Surg. Gynec. & Obst., 1961, v112, 11.
3. Fisher, E. R., Fisher, B., Cancer Res., 1961, v21, 275.
4. Fisher, B., Fisher, E. R., Ann. Surg., 1959, v150, 731.
5. ———, Cancer, 1961, v14, 547.
6. ———, Cancer Res., 1966, v26, 183.
7. Fisher, E. R., Fisher, B., Cancer, 1959, v12, 926.
8. Fisher, B., Fisher, E. R., Surg. Gynec. & Obst., 1966, v122, 791.
9. Rajam, P. C., Jackson, A. L., Black, S. H., J. Lab. & Clin. Med., 1958, v51, 767.
10. Selecki, E. E., Australian J. Exp. Biol. & Med. Sci., 1959, v37, 489.

Received November 9, 1966. P.S.E.B.M., 1967, v124.