patients with MS does not imply or indicate a direct relationship but warrants continuing investigation.

1. Tyler, H. R., *Medicine,* 1957, v36, 147.
2. McAlpine, D., Compston, N. D., Lumsden, C. E., *Multiple Sclerosis,* E. & S. Livingston Ltd., Edinburgh and London, 1955.
3. Courville, C. B., *Bull. L. A. Neuro. Soc.,* 1956, v21, 1.
4. Noad, K. B., *Med. J. Aust.,* 1960, v2, 881.
5. Ruckle, G., Rogers, K. D., *J. Immunol.,* 1957, v78, 341.
6. Shaffer, M. F., Rake, G., Hodes, H. L., *Am. J. Dis. Child.,* 1942, v64, 815.
7. Kabat, E. A., Landow, H., Moore, D. H., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 260.
8. Ivers, R. R., McKenzie, B. F., McGuckin, W. F., Goldstein, N. P., *Brit. Med. J.,* 1960, v176, 515.
9. Bauer, H., *World Neurology,* 1961, v2, 254.
10. Herriott, R. M., *Science,* 1961, v134, 256.
11. Rustigian, R., *Virology,* 1962, v16, 101.
12. Tawara, J. T., Goodman, J. R., Imagawa, D. T., Adams, J. M., *Virology,* 1961, v14, 410.
13. Dulbecco, R., Vogt, M., Strickland, A. G. R., *ibid.,* 1956, v2, 162.
14. Greenfield, J. G., Blackwood, W., McMenemey, W. H., Meyer, A., Norman, R. M., *Neuropathology,* Edward Arnold Ltd., London, 1958.
15. Kennedy, C., Carter, S., *Pediatrics,* 1961, v28, 377.
16. Adams, D. K., Sutherland, J. M., Fletcher, W. B., *Brit. Med. J.,* 1950, v2, 431.
17. Adie, W. J., *ibid.,* 1932, v2, 997.
18. Benedict, W. L., *Proc. Mayo Clin.,* 1933, v8, 147.
19. Ford, F. R., *Diseases of the Nervous System in Infancy, Childhood and Adolescence,* 3rd Ed., Chas. Thomas, Springfield, 1952.
20. Capolongo, G., *Arch. di Ottalmol.,* 1951, v55, 71.

Received July 16, 1962. P.S.E.B.M., 1962, v111.

Metabolism of Cl^{36}-Dichloromethotrexate by Transplantable Liver Tumors. (27856)

RICHARD H. ADAMSON, TI LI LOO, AND HAROLD P. MORRIS
National Cancer Institute, National Institutes of Health, Bethesda, Md.

Dichloromethotrexate* (DCM), a folic acid antimetabolite, has been reported to be metabolized in several animal species(1). Recently such a metabolic change was also observed to occur *in vitro* by an enzyme system localized in the soluble (72,000 × *g* supernatant) fraction of liver(2). The metabolite† of DCM is about 1000 times less active in inhibiting folic reductase than the parent compound(3).

An ideal agent for treatment of hepatic tumors might be a carcinostatic compound which (i) accumulates in liver, (ii) is metabolized rapidly by normal liver to a less toxic form and (iii) is not metabolized by the tumor(4). Because DCM is bound to liver tissue(1), is rapidly metabolized by normal liver, and probably "detoxified" by this mechanism it was thought desirable to study the *in vitro* metabolism of DCM by a variety of transplantable liver tumors to determine if the above requisites were fulfilled. Another objective of the present study was further to characterize these tumors as to the extent of enzymatic deviation from normal liver.

Methods. The liver neoplasms used in this study were carried intramuscularly or subcutaneously in male rats in our laboratories. Transplantable tumors studied included the Novikoff hepatoma, tumor 5123, 5123 TC, hepatoma 3683, hepatoma 7800 and the Reuber (H-35) hepatoma. The Novikoff tumor was carried in Holtzman rats; tumors 5123, 5123 TC and 7800 were carried in Buffalo rats; and the 3683 and H-35 tumors were car-

*N-{3,5-dichloro-4-[(2,4-diamino-6-pteridinylmethyl)-methylamino] benzoyl}-glutamic acid is the chemical name. The abbreviated generic name, DCM, is used throughout the text.

† The metabolite has tentatively been identified as 4-deamino-4,7 dihydroxy DCM (generic name); N-{3,5-dichloro-4[2-amino-4,7 dihydroxy-6-pteridinylmethyl)-methylamino]benzoyl}-glutamic acid is the chemical name.

TABLE I. Metabolism of Cl^{36}-DCM* *in vitro* by Normal Liver and Transplantable Liver Tumors.

Tumor	No. of exp	Metabolism in μmoles metabolite formed/g tissue used
Normal liver†	7	1.28 ± .07
Novikoff tumor	6	.00
3683 "	6	.22 ± .05
7800 "	4	1.18 ± .09
5123 TC "	8	1.20 ± .11
5123 "	7	1.27 ± .16
Reuber " (H-35)	4	1.39 ± .12

* Homogenates equivalent to 1.0 g of tissue were incubated with 1.99 μ moles of Cl^{36}-DCM for 3.5 hr aerobically in 0.1 M Tris buffer pH 8.2.

† Sprague-Dawley rats; values obtained with Holtzman, Buffalo and ACI rats were similar. Values in table are averages ± standard deviations.

ried in ACI/N rats. Morphological characteristics of some of these neoplasms have been published(5,6,7). Some of the biochemical characteristics of these tumors have been summarized by Potter(8).

Animals were killed by a blow on the head and the livers or tumors were excised immediately. Tumors were dissected free of necrotic and supporting connective tissue and rinsed with cold isotonic KCl. The tissue was then homogenized in 3 parts of cold isotonic KCl with a Potter homogenizer having a plastic pestle. Although the enzyme is localized in the soluble fraction of normal liver, homogenates were used in assaying the enzyme activity because of the possibility that intracellular distribution of the enzyme system may be different in the tumor cell.

Assay of enzyme activity was carried out as previously reported(2). A measured amount of liver or tumor tissue was placed inside a cellulose sac in a flask containing Cl^{36}-DCM(9) dissolved in 0.1 M Tris buffer [tris-(hydroxymethyl)-aminomethane] of pH 8.2 and incubated aerobically with shaking at 37° for 3.5 hours. At the end of incubation qualitative estimation of metabolism was made by differential spectrophotometry, using a blank containing no substrate. For quantitative assay of the metabolite the following technic was used. Inside solutions (denatured by heating at 100°C for 5 minutes then centrifuged) and outside solutions were examined by high voltage electrophoresis at pH 10 followed by autoradiography and counting of the radioactivity in all spots(1,9). The metabolite could be separated from the parent compound by use of this technic. Recovery of added radioactivity was 90%.

Results and discussion. The ability of various hepatic tumors to metabolize Cl^{36}-DCM is summarized in Table I. The Novikoff hepatoma lacked the ability to metabolize DCM. The ability of the 3683 tumor to metabolize DCM was detectable only by use of the electrophoresis and radioautograph technic, *i.e.*, spectrophotometric examination of outside solutions after incubation failed to reveal any metabolite. Metabolism of DCM by the Morris tumor 5123, 5123 TC, 7800 and the Reuber tumor was comparable to normal liver.

The growth rate (time of inoculation until death) of the various tumors varies from hepatoma 5123 (60-90 days) through hepatoma 7800, Reuber, 5123 TC, 3683, to Novikoff tumor (7 days). The 2 transplantable neoplasms with low (or not detectable) enzyme activity in metabolizing Cl^{36}-DCM, *i.e.*, the Novikoff tumor and 3683 hepatoma were rapidly growing tumors. However, the less rapid growing "minimal-deviation" tumors(8) retained the ability to metabolize DCM. It has been shown that parallel with the increasing growth rate the cellularity of the tumors increased whereas the nitrogen content declined(10).

Previous reports indicate that even the Morris tumor 5123 lacked the ability to metabolize certain drugs(4). However, it should be emphasized that those enzyme systems were localized in the liver microsomal fractions whereas the present enzyme system is localized in the soluble fraction.

Since DCM is metabolized to a less active folic acid antagonist by "minimal-deviation" hepatomas it is probable that DCM would be of little chemotherapeutic value in treatment of such tumors. Preliminary investigations have shown that DCM did not increase the life span of rats bearing the Morris 5123 tumor. However, the possibility remains that DCM may be of some value in treatment of rapidly growing hepatic tumors that do not metabolize it. Studies of the chemotherapeutic efficacy of DCM in Novikoff tumor as

compared with Morris hepatoma 5123 are in progress.

In the search for drugs effective against hepatic tumors one must take into account the fact that a drug may be active against some types of liver hepatomas but inactive against others. Thus, a screening program for drugs effective against hepatomas should include both the malignant slow growing "minimal-deviation" tumors and the rapidly growing "multiple deviation"(8) tumors.

Summary. Several types of transplantable hepatomas were assayed for their ability to metabolize Cl^{36}-DCM *in vitro*. The Novikoff tumor lacked the ability to metabolize DCM. The tumor 3683 had a slight amount of enzyme activity. Other tumors assayed, *i.e.*, 5123, 7800, 5123 TC and Reuber hepatomas had activity similar to normal liver. Possible therapeutic implications of these findings were discussed.

The authors wish to thank Miss Maggie J. Earle for technical help in enzyme assays.

1. Oliverio, V. T., Davidson, J. D., *J. Pharmacol. Exp. Therap.*, 1962, v137, 76.

2. Loo, T. L., Adamson, R. H., *Biochem. Pharmacol.*, 1962, v11, 170.

3. Misra, D. K., personal communication.

4. Adamson, R. H., Fouts, J. R., *Cancer Research*, 1961, v21, 667.

5. Novikoff, A. B., *ibid.*, 1957, v17, 1010.

6. Morris, H. P., Sidransky, H., Wagner, B. P., Dyer, H. M., *ibid.*, 1960, v20, 1252.

7. Reuber M. D., *J. Nat. Cancer Inst.*, 1961, v26, 891.

8. Potter, V. R., *Cancer Research*, 1961, v21, 1331.

9. Oliverio, V. T., Davidson, J. D., *J. Pharmaceut. Sci.*, 1961, v50, 353.

10. Weber, G., Morris, H. P., *Proc. Am. Assn. Cancer Res.*, 1962, v3, 370.

Received October 4, 1962. P.S.E.B.M., 1962, v111.

Transfer of Experimental Auto-Immune Nephrosis in Rats.* (27857)

WALTER HEYMANN, JANET L. P. HUNTER, DONALD B. HACKEL,† AND FRANCIS CUPPAGE

Departments of Pediatrics and Pathology, Western Reserve School of Medicine, Cleveland, Ohio

It has been reported that repeated intraperitoneal (i.p.) injection of rat kidney suspensions incorporated in Freund's complete adjuvants resulted in severe nephrotic renal disease in 87% of 92 rats(1,2). The following observations suggested an autosensitization to rat kidney proteins as the underlying pathogenetic mechanism: (a) The injection of muscle, lung or intestinal mucosa obtained from rats failed to produce renal disease(2); (b) renal cortex proved to be more antigenic than renal medulla; (c) ACTH and cortisone prevented and nitrogen mustard (H_2N) significantly reduced disease incidence(2); (d) complement activity in serum decreased simultaneously with development of proteinuria(3); (e) precipitating antibodies to rat kidney proteins were detected in sera and ascitic fluid, using double diffusion agar plates according to Ouchterlony, and antibodies in sera were also noted, using hemagglutination technics(3).

It is reported here that renal disease thus produced can be transferred to healthy rats by 2 different methods: (a) by parabiosis, and (b) by i.v. injection of emulsions of spleens of rats with incipient renal disease into litter mates treated at birth with emulsions of spleen obtained from other rats of the same litter.

Methods and procedures. Unless otherwise stated, methods and procedures were the same as previously described(1,2,3). The only change in procedures pertaining to disease production was that tissue suspensions

* Supported by grant from Nat. Inst. of Health, P.H.S. and from Am. Heart Assn., Inc.

† Now at Duke University, School of Med., Dept. of Pathology, Durham, N. C.