acting with other antigens of the kidney(11, 12). Lung also contains antigens capable of reacting with kidney localizing antibody but does not fix them efficiently *in vivo*(13).

The enlargement of the adrenal gland of rats injected with nephrotoxic serum although showing no histological damage(2) is probably a manifestation of increased permeability of the cortical sinusoids in this organ caused by the extensive localization of the heterologous antibody in these sites.

Summary. The site of fixation in the adrenal gland of rat kidney antibody administered *in vivo* has been demonstrated by the immunohistochemical technic to be along the vessels and sinusoids of the cortex. There is no fixation in the medulla although the medulla does contain antigen capable of reacting with the antibody *in vitro*. The possible derangement of adrenal function may be due to effect of the anti-kidney antibodies.

1. Bale, W. F., Spar, I. L., *J. Immunol.*, 1954, v73, 125.

2. Lippman, R. W., Marti, H. U., Jacobs, E. E., *A.M.A. Arch. Path.*, 1952, v54, 169.

3. Seegal, B. C., Loeb, E. N., *J. Exp. Med.*, 1946, v84, 211.

4. Pressman, D., Yagi, Y., Hiramoto, R., *Int. Arch. Allergy and Applied Immunol.*, 1958.

5. Day, E. D., Planinsek, J., Korngold, L., Pressman, D., *J. Nat. Cancer Inst.*, 1956, v17, 517.

6. Hiramoto, R., Pressman, D., *Cancer Res.*, 1957, v17, 1135.

7. Coons, A. H., Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

8. Hiramoto, R., Engel, K., Pressman, D., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 611.

9. Hiramoto, R., Goldstein, M., Pressman, D., *Cancer Res.*, 1958, in press.

10. Maximow, A. A., Bloom, W., *A Textbok of Histology*, 4th ed., W. B. Saunders Co., Philadelphia, 1944, p318.

11. Cruickshank, B., Hill, A. G. S., *J. Path. and Bact.*, 1953, v66, 283.

12. Eisen, H. N., Pressman, D., *J. Immunol.*, 1950, v64, 487.

13. Eisen, H. N., Sherman, B., Pressman, D., *ibid.*, 1950, v65, 543.

Received June 2, 1958. P.S.E.B.M., 1958, v98.

Rapid Method of Metabolically Characterizing Individual Tumors.* (24213)

STANFIELD ROGERS AND BARNES WOODHALL

University of Tennessee Memorial Research Center and Hospital and Division of Neurosurgery, Duke University School of Medicine.

Characterization of neoplasms as to class by usual methods of pathology is well known to have but limited value in selection of chemotherapeutic agents for treatment of individual tumors. Previous studies have shown that the chromatographic spectrum of metabolic constituents of crude animal tissues often yielded highly specific information allowing for laboratory differentiation of disease processes appearing the same with the light microscope(1). With this in mind a variety of glial tumors together with a number of tumors metastatic to the brain were chromatographed and the patterns obtained after spraying with ninhydrin recorded. In addition to this, fresh tumor tissues were studied individually in relation to amino acids which they took out of a synthetic media of known composition. In this report only the tumors morphologically classed as glioblastoma multiforme will be considered.

Materials and methods. Tumors were obtained in the fresh within a few minutes after surgical extirpation. Representative portions of the tumor were sent to the neuropathology laboratory for the usual pathologic study and diagnosis. Other representative parts were cut into small fragments, using a dissecting microscope to eliminate necrotic tissue as far as possible, squeezed into sheets of Whatman No. 1 chromatographic paper, dried in a hood at room temperature and chromatographed in

* Supported in part by P.H.S. Grants and Duke University Center for Study of Aging.

butanol acid water (40 : 10 : 50) solvent system using the ascending method. The details of this technic have been previously described (1). After 18 to 20 hours the developed chromatograms were dried and sprayed with 0.4% ninhydrin and acetone, heated in an incubator at 70° for 10 to 15 minutes, and the patterns derived in the individual tumor studied and recorded photographically. Other representative pieces of fresh tumor were cut into tiny fragments, again special care being taken to eliminate visible necrotic tissue with the dissecting microscope, and about 50 mg of the minced tumor tissue placed in 3 cc of Difco culture medium TC 199 and thereafter incubated for 24 hours at 37°C. Following incubation, the cells were sedimented by centrifugation and supernatant taken for analysis. Two to 3 parallel tests were run on each tumor. 0.01 cc of media from each run of individual tumors were put alternately along the base line of Whatman No. 1 chromatographic paper sheet intermixed with these was 0.01 cc of media never exposed to tumor cells. After drying, the sheets were developed in butanol acetic acid solvent for 18 to 20 hours, dried and sprayed with 0.4% ninhydrin and acetone, as above. With this technic the following amino acids may be generally separated: glutamic acid, phenylalanine, tyrosine, methionine, alanine, histidine, leucine, threonine, lysine and cystine. The strips may be judged visually for very rough quantitative estimation of amount of amino acids present, as judged from density and size of individual spots or may be much more sensitively judged through examination in an analytical densitometer. This latter method was mainly used. The results of the study of the media after exposure to the tumor cells provided a further control as to any influence of necrosis of the cells upon the pattern of uptakes. In occasional flasks new ninhydrin positive substances not present in the original media were seen. In such instances the uptakes of certain of the amino acids by the individual tumor were hidden. In some of these the flask contained a larger amount of amino acids than was present in the original media. Such flasks did not figure in the results. As might be expected there was some variability in the amounts of individual amino acids taken up by the individual tumor from flask to flask. In every instance however, those taken up in the largest amounts always repeated though the amino acids taken up in proportionately smaller amounts might be missed. The same result generally held in those tumors whose uptakes were measured on separate occasions. An example of this is the comparison of tumor 11 with tumor 34 which was taken from the same individual with an interval of several months between. In studies of 3 tumors in the group of 12 being reported, an amount of ethionine equivalent to the amount of methionine originally in the media was added to test whether the influence of the analogue might exert a measurable effect on amino acid uptakes of tissue under study.

Results. In tumors numbered 1 through 11, individual tumors were tested only as to whether they took up amino acids at all, rather than quantitatively. Furthermore, studies of these tumors did not include a parallel test of the ninhydrin positive pattern of the constituents of the crude tumor. The remainder of the tumors were studied quantitatively and in relation to the ninhydrin pattern of crude tissue. In tumors numbered 17 through 39 the percentages indicate the amount of individual amino acids removed from the media by tumor cells during 24 hour incubation. Though it is evident that these various glioblastomas have much in common, particularly as to utilization of glutamic acid, tyrosine, phenylalanine, leucine and methionine, individual variation from tumor to tumor as to relative proportions of various amino acids used, and in some instances utilization of certain different amino acids, for example alanine, histidine and lysine, appears the rule rather than the exception. Similar relationships were found upon comparison of patterns obtained in chromatographing the whole tumor tissue. In Fig. 1 a rather remarkable similarity is seen between the pattern in tumor 19 on comparison of tumor 35, though certain differences are still evident. That the amino acid uptakes of these 2 tu-

TABLE I. Glioblastoma.

Tumor	Glutamic	Phenyl-alanine	Tyrosine	Methio-nine	Alanine	Histidine	Leucine	Lysine	Cystine	Ethionine effect
	(% uptake)									
1	×	×	×					ine.		
3	×									
5	×									
10	×	×	×	×	×	×				
11	×	×								
17	40	20	50		50		30			
19	20		15	20			10			××××
22	50									
30			50	30			20			×××
34	50	20	20			30				none
35	30	15	20	20			10			
39	15						15	30		

mors also had great similarity is noteworthy (Table I). The chromatographic pattern obtained from tissue of tumor 17 and tumor 34 showed a much more considerable difference both in relation to each other and to tumors 19 and 35 (Fig. 1). It is pertinent that the relative proportion of various amino acids taken up by tumors 17 and 34 was quite different, and further that one of these tumors, 17, took large amounts of alanine from the media, and 34, large amounts of histidine (Table I). Both these findings are in contrast to the general run of glioblastomas. The pattern found in tissues of tumor 39 was also highly individual (Fig. 1). This might have been expected from its relative amino acid uptakes as well as its utilization of a large amount of lysine. In tumor No. 19 the presence of ethionine completely prevented uptake of any of these amino acids as measured by this system. In tumor No. 30 the uptake was almost entirely blocked. In contrast in tumor No. 34 the ethionine had no effect whatever upon the uptake of various amino acids. It is noteworthy that the 2 tumors, 19 and 30, had similarities both as to amino acid uptake and as to their tissue chromatographic pattern. It is also pertinent that tumors 19 and 30 utilized methionine quite extensively in contrast to tumor 34 in which no utilization was discernible. In view of the fact that ethionine is the metabolic antagonist to methionine and, in these 3 tumors, the ones in which methionine was used were the ones in which ethionine was effective, it would seem worthwhile to find whether the specific antagonist of amino acids utilized by an individual tumor might be a chemotherapeutic agent of choice to treat the individual tumor.

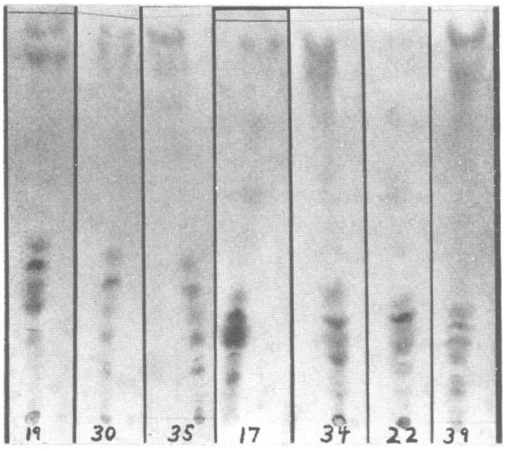

FIG. 1. Comparison of chromatographic patterns of several glioblastomas as shown with ninhydrin following development in butanol acetic acid water solvent system.

It would appear from these results that the combined studies of individual tumors by means of technics of classic pathology, study of the chromatographic pattern of individual tumors, and study of metabolic requirements of the same individual tumors will yield useful information leading both to understanding of the metabolic difference between normal and neoplastic cells and lead as well to a more effective utilization of chemotherapeutic agents.

Summary. A rapid method for biochemically characterizing individual neoplasms particularly in relation to amino acid content and requirements is described. It is suggested

that use of this method will facilitate the more accurate choice of chemotherapeutic agents in treatment of individual tumors even of the same morphologic class, and perhaps be an effective means of rapidly screening metabolic antagonists as to their effect upon individual tumors.

1. Rogers, S., Berton, W. M., *Lab. Invest.*, 1957, v6, 310.

Received June 6, 1958. P.S.E.B.M., 1958, v98.

Effect of Beta-Carotene and Cortisone on Vaginal Epithelium of Vitamin A Deficient Rats.* (24214)

EDWIN G. RICE† AND WALTER J. BO (Introduced by C. J. Hamre)
Department of Anatomy, University of North Dakota School of Medicine, Grand Forks

Conversion of beta-carotene to Vit. A is partially influenced by tocopherols, Vit. B_{12}, antibiotics, phosphates, insulin and thyroxin (1). Recently Clark and Colburn(2), utilizing biochemical analyses, found that administration of cortisone to rats on stock laboratory diet or Vit. A free diet caused rapid reduction in quantity of Vit. A in liver and kidney. They further demonstrated that only when large amounts of beta-carotene were administered to cortisone-treated rats was there adequate conversion of beta-carotene to Vit. A and deposition of Vit. A in liver and kidney. They suggested that conversion of beta-carotene to Vit. A is impaired in cortisone-treated rats.

The results obtained by Clark and Colburn (2) seem to indicate that cortisone-treated animals would eventually become Vit. A deficient and exhibit manifestations of the deficiency. However, it is also possible that blocking of the conversion of beta-carotene to Vit. A by cortisone may result in quantitative reduction of the Vit. A stored in body depots, and may not produce pathological changes in organs sensitive to Vit. A deficiency. With this question in mind, and by utilizing cornification of the vaginal epithelium of the rat as criterion of Vit. A deficiency(3), the present investigation was undertaken to determine whether beta-carotene in the presence of cortisone treatment would cause cornification of vaginal epithelium of Vit. A deficient rats to disappear.

Methods. Fifty-one rats of Wistar strain were placed on a Vit. A free diet‡ on day 20 of life. Daily weighings and vaginal smears were begun 3 weeks after initial feeding of Vit. A free diet.

When the Vit. A deficient animals exhibited vaginal cornification continuously for 3 to 4 days, they were subjected to the following procedure. In Group I (17 animals), the rats received daily either 6, 12, 24, 48, or 480 μg of beta-carotene§ orally for 10 days. Rats of Group II (6 animals) received 2.5 mg of cortisone acetate subcutaneously/day for 10 days but received no beta-carotene. Animals in Group III (19 animals) for 10 days received daily subcutaneous injections of 2.5 mg of cortisone acetate and in addition either 12, 24, 48, or 480 μg of beta-carotene orally. Rats in Group IV (9 animals) received 2.5 mg of cortisone acetate subcutaneously/day for 3 days prior to dual treatment of cortisone and beta-carotene daily for the next 10 days. Dosages of beta-carotene used for animals of Group IV were 12, 24, or 48 μg.

At the end of prescribed treatment, the animals were sacrificed and the vagina of each animal removed and fixed in Zenker's solution. After fixation the tissue was dehydrated, imbedded in paraffin, and sectioned at

* Supported by grant from Nat. Vit. Found.

† Student Part-time Research Fellow of U.S.P.H.S., Division of Nat. Inst. of Health.

‡ Vit. A Test Diet U.S.P., Nutritional Biochemicals Corp., Cleveland, O.

§ The beta-carotene was supplied through the courtesy of Hoffmann-LaRoche, Nutley, N. J.