of the rubella virus-induced CVR in the same cell, for no interferon-like substances appear to be elaborated by BS-C-1 cells infected with rubella virus(5). No explanation for these differences can currently be made.

Experiments reported elsewhere have confirmed the utility and relative simplicity of the BS-C-1 interference system for the isolation of dengue viruses of several types from human serum, and for performing virus neutralization tests to identify the agents isolated (5). The current experiments define optimal times for harvesting virus and for detecting interfering agents by challenge. Seed viruses for neutralization tests, containing minimal amounts of interferon, can be obtained by harvesting washed cell sheets on the fourth or fifth day of incubation.

Summary. Infection of BS-C-1 cells by dengue virus produces resistance to challenge with polio virus type II. The interference is related to the production of an interferon which is released into the media following the time when maximal intracellular dengue virus titers are present. The interferon appears similar to other mammalian interferons and can be separated from the infectious virus by gel filtration with Sephadex G-200. The use of this method for isolation and identification of dengue viruses is discussed.

1. Hotta, S., Evans, C. A., Proc. Soc. Exp. Biol. and Med., 1956, v93, 53.
2. Halstead, S. B., Sukhavachana, P., Nisalak, A., ibid., 1964, v115, 1062.
3. Parkman, P. D., Buescher, E. L., Artenstein, M. S., McCown, J. M., Mundon, F. K., Druzd, A. D., J. Immunol., 1964, v93, 595.
4. Vilcek, J., Acta Virologica (Prague), 1960, v4, 308.
5. Russell, P. K., Buescher, E. L., McCown, J. M., Ordonez, J., Amer. J. Trop. Med. and Hyg., in press.
6. Reed, L. J., Muench, H., Am. J. Hyg., 1938, v27, 493.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., J. Biol. Chem., 1951, v193, 265.
8. Isaacs, A., Adv. Virus Res., 1963, v10, 1.
9. Wagner, R. R., Ann. Rev. Microbiol., 1963, v17, 285.
10. Merigan, T. C., Science, 1964, v145, 811.
11. Wheelock, E. F., Sibley, W. A., Lancet, 1964, v1, 382.
12. Lampson, G. P., Tytell, A. A., Nemes, M. M., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1963, v112, 468.
13. Vilcek, J., Acta Virologica (Prague), 1962, v6, 144.

Received March 4, 1966. P.S.E.B.M., 1966, v122.

Effect of Partial Hepatectomy and Pregnancy on Tumor Growth and Alanine-α-Ketoglutarate Transaminase Activity.* (31191)

HOMER R. HARDING,† FRED ROSEN, AND CHARLES A. NICHOL
Department of Experimental Therapeutics, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, N. Y.

Characteristic of tumor growth is the competition between the neoplasm and normal tissues for the nutritional resources of the host(1,2). Emaciation and alterations in metabolism occur in animals bearing rapidly growing tumors. Previous studies in our laboratory demonstrated that hepatic alanine transaminase activity is markedly depressed in adult rats bearing Walker carcinoma 256 (3). It was of interest, therefore, to determine if this effect on a transaminase enzyme would also occur under other conditions which involve the growth of new tissues, such as during pregnancy and liver regeneration. The rate of tumor growth and changes in alanine transaminase activity were also observed when the Walker tumor was growing in competition with regenerating liver or rapidly growing fetal tissues. Furthermore, the induction of alanine transaminase by cortisol

* Supported in part by Grant CA-05671 from Nat. Cancer Inst.

† Present address: Sterling-Winthrop Research Institute, Rensselaer, N. Y.

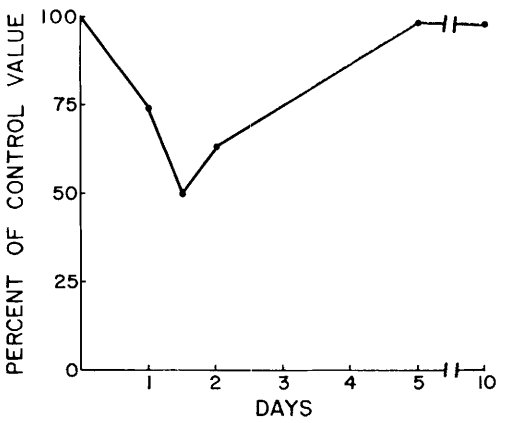

FIG. 1. Effect of partial hepatectomy on liver alanine transaminase activity.

was studied in both liver and tumor in each of these experiments.

Materials and methods. Partial hepatectomy of male Holtzman rats weighing approximately 150 g was performed by the technique of Higgins and Anderson(4). Virgin rats weighing 230 to 260 g were bred for the pregnancy studies. The day of conception was determined by the presence of sperm in vaginal smears. A sample of the vaginal contents (obtained by inserting a flat metal probe) was taken every morning; the probe was then gently shaken in a drop of saline and the suspension examined for sperm under low power magnification.

The Walker tumor was cut into 1 mm cubes under aseptic conditions and implanted by trochar into the right lateral flank of each animal. Samples of liver and tumor tissue were assayed for alanine-α-ketoglutarate transaminase activity by a fluorometric technique previously described(5). Protein content was determined by a modification of the Folin method(6).

Results. The average level of alanine transaminase in the residual liver of 3 rats following partial hepatectomy is shown in Fig. 1. Thirty-six hours following the operation, alanine transaminase activity in the regenerating tissue was about 50% that found in the liver removed at time of operation. Regeneration of liver mass is complete 5 days following partial hepatectomy and at this time the activity of alanine transaminase in the regenerated liver was equal to the control value for the sample of liver excised at the time of operation.

Treatment with cortisol markedly enhances alanine transaminase activity in rat liver. Small increases in enzyme activity have been observed 24 hours following steroid injection and after 2 days or with further treatment, greater increases in transaminase activity were observed(5,7). On this basis, 2 injections of cortisol (2 mg) were administered on the 12th and 24th hours following hepatectomy and the regenerating liver was assayed after 48 hours. Treatment with a total of 4 mg of cortisol did not significantly increase alanine transaminase activity in the regenerating liver (Fig. 2), whereas similar treatment of intact rats almost doubled the activity of this enzyme.

The effect of partial hepatectomy on the growth of the Walker tumor is shown in Table I. At the time of operation, the weight of the Walker tumor averaged 2.3 g. After 15 days, the tumors growing in the partially hepatectomized animals weighed about 11 g and were only 40% the size of the tumors grown in the control animals. In addition to the restrictive effect on the growth of the

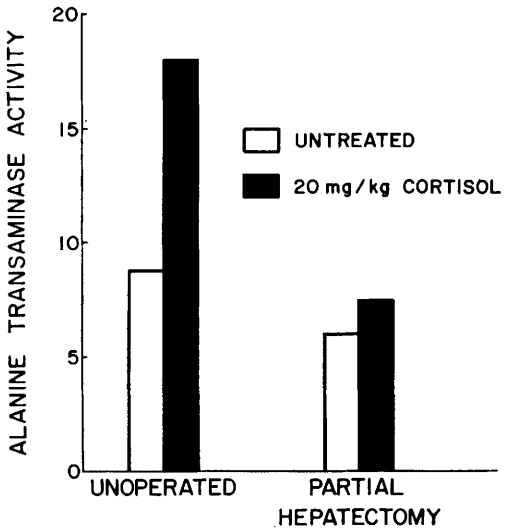

FIG. 2. Lack of response of liver alanine transaminase to cortisol in partially hepatectomized rats. Enzyme activity is expressed as mMoles of substrate utilized per gram protein per hour. Mean values for 5 rats in each group are given. The livers were taken for assay 48 hr after operation and 24 hr after second dose of cortisol was given.

TABLE I. Alanine Transaminase Activity and Tumor Growth in Partially Hepatectomized Rats.

	Liver		Walker tumor	
	Wt	Transaminase§	Wt	Transaminase§
Control*	5.39 ±1.4 ‡	5.61 ± .84	—	—
Hepatectomy	7.21 ± .97	4.56 ± .36	—	—
Walker tumor	7.49 ± .82	5.13 ± 1.6	25.2 ± 11	.134 ± .088
Hepatectomy + Walker tumor†	3.93 ± .68	10.3 ± 3.9	10.9 ± 1.2	.141 ± .048

* Sample of liver removed at time of hepatectomy on 10th day.
† Walker tumor was implanted on day 1 and hepatectomy done on day 10; all rats were sacrificed on day 15.
‡ Mean values for 5 rats per group ± standard deviation.
§ Millimoles of substrate utilized per g protein per hr.

tumor, the rate of growth of the liver in the hepatectomized tumor-bearing animals was decreased so that liver weights, usually normal 5 days post-operative, were still about 50% below normal at this time.

Previously, it was shown that alanine transaminase activity in the Walker tumor was increased significantly (15- and 3-fold) when tumor growth was inhibited by administration of cortisol or by feeding a high protein diet (8). Starvation or alloxan diabetes also markedly impaired tumor growth, but neither of these conditions resulted in an increase in the transaminase activity in the tumor. Similarly, the activity of this enzyme in the Walker tumor was unaltered when growth of this neoplasm was retarded in the partially hepatectomized rats (Table I). In contrast, the level of alanine transaminase was increased in the livers of partially hepatectomized rats bearing tumors. The increase in body weight of these rats during the 15-day experimental period was only about 30% less than that of the tumor-bearing or partially hepatectomized animals. The elevated hepatic alanine transaminase values may be related to the greater degree of stress and presumably higher levels of blood corticosterone in these animals.

A more prolonged period of competition between rapidly growing tissues was achieved by growing the Walker tumor in pregnant animals. In these experiments, the Walker tumor was implanted on the fifth day of gestation and the livers and tumors removed on the twenty-first day of pregnancy (Table II). Transplanting the Walker tumor into pregnant rats on the fifth day of gestation did not markedly affect the average litter size (9.5 fetuses), or fetal weight (5.6 ± 1.0 g, standard deviation) when compared with non-tumor bearing rats (average litter size—11.8 fetuses weighing an average of 3.8 ± 0.5 g, standard deviation). However, the growth of the Walker tumor was markedly impaired during pregnancy. Thus, the tumors removed from the pregnant rats were only one-fifth the weight of those grown in the non-pregnant animals.

Treatment of non-pregnant rats with the steroid for 10 days inhibited tumor growth by more than 90% (Table II). Although the rate of growth of the tumor in pregnant rats was much slower, administration of cortisol to these animals did cause a significant inhibition ($P<.01$) of tumor growth. When non-pregnant tumor-bearing rats were treated with cortisol, alanine transaminase activity was increased 2- to 3-fold in the liver and the activity of this enzyme was increased 5-fold in the tumor. In pregnant animals cortisol administration did not significantly increase liver alanine transaminase activity, confirming an earlier observation by Curry and Beaton(9); the activity of this enzyme in tumor also was unresponsive in pregnant animals. The retardation of tumor growth which occurred during the period of fetal growth was not associated with any significant change in the activity of this transaminase in tumor (Table II).

Discussion. Observations of relatively low alanine transaminase activity in rapidly growing tissues led Cohen and Hekhuis(10) and Braunstein(11) to postulate that there was an inverse relationship between transamina-

TABLE II. Alanine Transaminase Activity and Tumor Growth in Pregnant Rats.

	Days of tumor growth	Tumor wt (g)	Alanine transaminase activity (millimoles of substrate utilized per g protein per hr) Tumor	Liver
Non-pregnant				
No treatment	—	—	—	22.1 ± 2.3‡
Cortisol, 12 mg/kg × 10 days	—	—	—	45.7 ± 7.1
No treatment	15	10.3 ± 5.6	.25 ± .08	14.5 ± 2.6
Cortisol, 12 mg/kg × 10 days*	15	.76 ± .23	1.34 ± .69	45.9 ± 7.1
Pregnant				
No treatment	—	—	—	7.6 ± 1.7
Cortisol, 12 mg/kg × 10 days	—	—	—	10.1 ± 5.2
No treatment	15†	2.1 ± .3	.40 ± .16	7.8 ± 3.6
Cortisol, 12 mg/kg × 10 days*	15†	1.2 ± .4	.47 ± .21	11.1 ± 3.0

* Cortisol administered subcutaneously daily for the last 10 days of tumor growth.
† Walker tumor implanted on day 5 of gestation and harvested on day 21.
‡ Mean values for 6 rats per group ± standard deviation.

tion and protein synthesis. This concept is supported by the data obtained in this study, which demonstrate that the level of alanine transaminase in liver is decreased following partial hepatectomy and during pregnancy. Thus, the previous observation that the activity of this transaminase enzyme in liver is markedly decreased during the growth of Walker carcinoma 256(3) is apparently related to the metabolic changes associated with the growth of new tissue rather than to any specific effect of the tumor. It is particularly noteworthy that the usual response of this transaminase in liver to treatment with cortisol does not occur during recovery from partial hepatectomy, during rapid fetal growth and during the growth of a large tumor. At present, the alterations in metabolism which underlie these adaptive changes are unknown. It is conceivable that the increased requirement for protein precursors for the growth of new tissue results in a relative deficiency of amino acids in liver which limits the synthesis of enzymes induced by cortisol. This explanation is consistent with the observation that both cortisol and an adequate intake of protein are required to induce maximum increases in alanine transaminase activity in the liver of adrenalectomized rats (5).

Paschkis *et al*(12) observed that when Walker carcinoma 256 was implanted on the same day that partial hepatectomy was performed, tumor growth was greater than that which occurred in the intact control animals. Bly and coworkers(13) found effects on both gestation and the growth of the Walker tumor depending on the time relationship between conception and tumor implantation. In our experiments, when the tumor was implanted on the fifth day of pregnancy or when hepatectomy was performed 10 days after tumor implantation, the period during which the tumor normally grows rapidly coincides with the time of rapid liver regeneration or fetal development. Under these circumstances, tumor growth was markedly inhibited.

Lymphoid tissues, such as the thymus gland and certain neoplasms, which are responsive to glucocorticoids show marked increases in alanine transaminase activity following cortisol administration(14). That this effect is somewhat specific is demonstrated by the finding that other conditions such as partial hepatectomy, pregnancy, starvation (8), and diabetes(8) which also cause regression of the Walker carcinoma 256 do not influence the alanine transaminase levels of this tumor. The inhibition of tumor growth during liver regeneration or pregnancy probably reflects the competition between tissues for essential nutrients, rather than a direct alteration in tumor metabolism such as may result from treatment with cortisol. Under each of these conditions, alanine transaminase was not inducible in the tumor by treatment with small doses of cortisol. Also, the regenerating liver and liver of the pregnant

rat do not show a rise in alanine transaminase activity after cortisol treatment. The factors which control the response of this enzyme in tumor and liver in partially hepatectomized and pregnant rats and the significance of enzyme induction in relation to the responsiveness of certain tissues to corticosteroids remain to be determined.

Summary. Consequences related to the metabolic requirements for growth of new tissues during fetal development and liver regeneration were studied with regard to: 1) activity of a transaminase enzyme, alanine-α-ketoglutarate transaminase; 2) growth of Walker carcinoma 256; and 3) response of the enzyme and the tumor to treatment with cortisol. The livers of pregnant or partially hepatectomized rats did not show an increase in alanine transaminase activity after administration of cortisol at doses which produced a significant response of this enzyme in normal control animals. The growth of the Walker carcinoma 256 in partially hepatectomized rats and pregnant animals was inhibited by 40% and 80%, respectively. While liver regeneration in tumor-bearing animals was retarded by 50%, the growing tumor had no observable effect on fetal development. Alanine transaminase activity was below normal endogenous levels in both regenerating liver and liver from pregnant rats.

1. Henderson, J. F., LePage, G. A., Cancer Research, 1959, v19, 887.
2. Mider, G. B., Tesluk, H., Morton, J. J., Acta Unio. Internat. Contra Cancrum, 1948, v6, 409.
3. Harding, H. R., Rosen, F., Nichol, C. A., Cancer Research, 1964, v24, 1318.
4. Higgins, G. M., Anderson, R. M., Arch. Path., 1931, v12, 186.
5. Rosen, F., Roberts, N. R., Nichol, C. A., J. Biol. Chem., 1959, v234, 476.
6. Lowry, O. H., Rosebrough, N. R., Farr, A. L., Randall, R. J., ibid., 1951, v193, 265.
7. Rosen, F., Harding, H. R., Milholland, R. J., Nichol, C. A., ibid., 1963, v238, 3725.
8. Rosen, F., Budnick, L. E., Solomon, D. K., Nichol, C. A., Cancer Research, 1961, v21, 620.
9. Curry, D. M., Beaton, G. H., Endocrinology, 1958, v63, 155.
10. Cohen, P. P., Hekhuis, L. G., Cancer Research, 1941, v1, 620.
11. Braunstein, A. E., Advances in Protein Chemistry, Academic Press, New York, 1947, v1.
12. Paschkis, K. E., Cantarow, A., Stasney, J., Hobbs, J. H., Cancer Research, 1955, v15, 579.
13. Bly, C. G., Drevets, C., Migliarese, J. F., Fed. Proc., 1955, v14, 399.
14. Rosen, F., Cancer Research, 1963, v23, 1447.

Received March 4, 1966. P.S.E.B.M., 1966, v122.

Mycoplasma granularum of Swine Origin as a Tissue Culture Contaminant. (31192)

JOSEPH G. TULLY
Laboratory of Bacterial Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

The problems of tissue culture contamination with mycoplasma, together with identification of species and source of isolates, have been reviewed recently(1,2). Most strains recovered have been of human origin, particularly *Mycoplasma hominis* type 1 and *M. orale* 1. Saprophytic *M. laidlawii* has been described as a contaminant on one occasion (3), as well as an avian strain, *M. gallisepticum*(4). The first suggestion that animal mycoplasma might be more extensively involved in tissue culture contamination occurred when agents recovered from cell cultures inoculated with human leukemic bone marrow(5) and spleen(6) were later identified as mycoplasma (strains Negroni(7) and 880 (8)) and subsequently typed as *M. pulmonis* (9,10), a serotype originally found in rats and mice. However, the recovery of *M. pulmonis* from uninoculated tissue cultures has not been reported and the impression that rodent strains are present in human tissues