

served a well defined increase in virus locally, indicating that in adult mice also some component(s) of the tail supports the growth of these viruses.

It has been pointed out that the FA strain of Theiler's virus differed from the GD VII strain in its failure to grow on tail tissue. It also showed a somewhat lower ID₅₀ titer throughout. Both of these viruses, however, require further study from certain standpoints, such as the optimum composition of the fluid medium, length of incubation, etc., before the influence of individual tissue explants can be fully evaluated.

Summary. In a fluid medium consisting of 3 cc of Simms' solution and 1.25 cc of ox-serum ultrafiltrate plus 0.25 cc of selected minced tissue in glucosol solution, the Col. SK, C(M) and M.M. viruses were found to grow freely on tissue explants of brain, lung, small intestine, heart muscle, and tail from

embryo mice, but not on explants of tongue skeletal muscle, kidney, liver, or spleen from the same embryos. Growth of all 3 viruses was also obtained on explants of minced 5-day chick embryos, but not to an equal degree.

The GD VII strain of Theiler's encephalomyelitis virus was carried through 15 culture passages on explants of brain and tail tissue, but failed to show well defined or continued growth on explants of other tissues and organs from embryo mice. Unlike the GD VII strain, the FA strain of Theiler's virus failed to grow on explants of tail tissue. Both strains grew on explants of chick embryo tissue, but the FA strain grew less freely than the GD VII strain, even on explants of mouse brain tissue, under the conditions described.

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Comparative Specific Gravities of Normal and Tumor Tissues.* (17767)

KANEMATSU SUGIURA AND ALVIN M. ARKIN.

From the Sloan-Kettering Institute for Cancer Research, Memorial Hospital, New York City.

Recently we observed that increased pressures were capable of inhibiting the growth of Sarcoma 180 in mice(1). This finding indicated the possibility that tumor tissue may have a different density from normal tissue. Preliminary studies on flotation of Sarcoma 180 in sodium chloride solutions appeared to support this possibility. Fawcett, Vallee, and Soule(2) have recently shown that blood cells sediment at different rates than cancer and mesothelial cells in albumin solutions.

In view of these findings, it was decided to investigate more systematically the specific gravity of various mouse, rat and human tu-

mors.

Materials and methods. The transplantable mouse tumors used in these experiments were Crocker sarcoma 180 Lewis sarcoma T241, sarcoma MA387, Patti myxosarcoma, mammary adenocarcinoma EO 771, Wagner osteogenic sarcoma, Ridgway osteogenic sarcoma, Patterson lymphosarcoma, Mecca lymphosarcoma, Harding-Passey melanoma and Andervont hepatoma. The original myxosarcoma was induced by methylcholanthrene, while the hepatoma was induced by *o*-aminoazotoluene. Sarcoma 180, myxosarcoma and the melanoma were grown in albino mice of the Rockland Farms strain; osteogenic sarcoma, lymphosarcoma and sarcoma MA 387 were grown in mice of the AKm strain (leukemic); sarcoma T241 and adenocarcinoma EO 771 were grown in C57 black mice in which they originated, and hepatoma was grown in strain C mice. The transplantable

* This work was supported by a grant made to the Sloan-Kettering Institute for Cancer Research by the American Cancer Society.

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2. Fawcett, D. W., Vallee, B. L., and Soule, M. H., *Science*, 1950, v111, 34.

rat tumors used in the present study were Flexner-Jobling carcinoma, Jensen sarcoma, Sarcoma R39 and Walker carcino-sarcoma 256. These tumors were grown in albino rats of the Sherman strain. Subcutaneous implantations of tumors (small pieces of tumor, each measuring about 1.5 mm³ and weighing approximately 6 mg) into healthy young animals (18-22 g mice; 100-125 g rats) were carried out by the usual trocar method (a single implant into the right axillary region).

The animals were given food (Purina Laboratory Chow) and water *ad libitum*. In our earliest observations on this phenomenon a very simple experiment was done. Sarcoma 180 was implanted into mice of the Rockland Farms albino strain in the usual axillary region. One week later the animal was killed with ether and pieces of tumor, liver, kidney, spleen, heart and skeletal muscle were dropped into a beaker of saline solution. The specific gravity of the solution was adjusted by adding salt or water, as necessary, until the tumor just floated. In such a solution, the

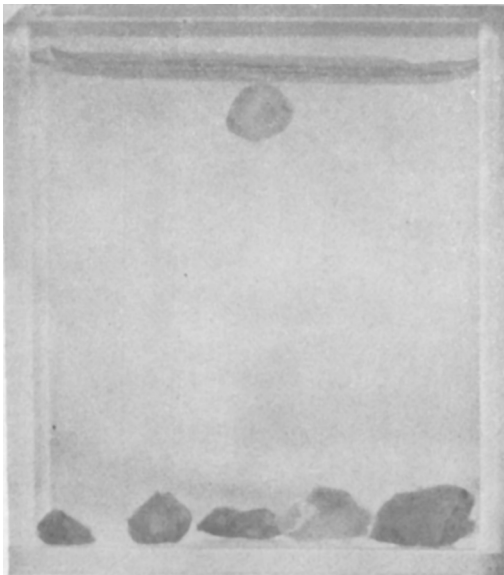


FIG. 1.

Sarcoma 180 floats in 8% sodium chloride solution. Heart, kidney, spleen, muscle and liver sink. (All tissues from the same mouse).

† Testis was even lighter than tumor, and would float in solutions in which Sarcoma 180 sank.

other tissues mentioned above[†] invariably sank to the bottom (Fig. 1). This behavior has been constant in several different batches of such tumorous mice tested at intervals during the last sixteen months.

More recently, the following technic was adopted for determining the specific gravity of tumor and normal tissues. After killing the tumor-bearing animal with ether anesthesia, the tumor was removed, and adherent fat and fascia cut away. A piece of tumor tissue 5 to 10 mm in diameter was cut from a healthy, non-necrotic area at the periphery of the tumor, quickly rinsed in 0.85% sodium chloride solution in water, and then touched to filter paper to remove the excess saline. It was then immediately dropped into a beaker of sodium chloride solution of known specific gravity (solutions of each percentage from 6% to 13% NaCl[‡] were available). The range of specific gravity was estimated by determining the density of solution in which the tumor would sink and in which it would float. A similar procedure was repeated with spleen, kidney, liver, heart, and thigh muscle of the host. All determinations were done within 10 to 30 seconds since the observations became unreliable after longer immersion in salt solution, the tissues becoming heavier, probably by absorption of salt.

At the time the specific gravity determinations were made, the tumor-bearing mice were about 2 to 4 months old and weighed about 20 to 35 g. The tumor-bearing rats were about 2 to 4 months old and weighed about 150 to 200 g. Sarcoma 180 was about 7 days after implantation; sarcoma T241, Ridgway osteogenic sarcoma, and both lymphosarcomas were about 2 weeks old; Wagner osteogenic sarcoma was about 3 weeks old; melanoma was about 6 weeks old; and hepatoma was about 10 weeks old. It is to be noted that these respective periods are the best age for tumor transplantation.

Results. The specific gravity of normal mouse tissues of various strains lies between

‡ The specific gravities of 6%, 8%, 10%, and 12% NaCl solutions at 20°/4°C. are 1.0413, 1.0559, 1.0707, and 1.0857, respectively.

TABLE I.
Flotation of Normal Mouse Tissues of Various Strains (% NaCl at 22°C).

Tissue	Rockland Albino		C57 Black		C ₃ H		AKm		C	
	S*	F*	S	F	S	F	S	F	S	F
Heart	9	10	9	10	9	10	9	10	9	10
Liver	11	12	11	12	11	12	11	12	11	12
Spleen	9	10	10	11	10	11	10	11	10	11
Kidney	9	10	9	10	9	10	9	10	10	11
Muscle	9	10	10	11	9	10	9	10	10	11

S = sink.

F = float.

the limits summarized in Table I. In all 3 tables, each determination summarizes determinations on 2 to 6 animals, which were practically uniform in every case. Heart and kidney were lightest, liver the heaviest tissue, in every animal. Although there are minor variations in the various strains of mouse, the pattern for each strain was quite constant. Specific gravities of normal tissues of male and female animals were essentially the same.

The specific gravities of certain transplantable mouse tumors are given in Table II. It will be noted that the tumor is always markedly lighter than any other tissues tested except in the case of the Harding-Passey melanoma and the Andervont hepatoma. Both of these tumors had specific gravities equivalent to that of a solution of 9 to 10% sodium chloride—comparable to some of the normal organ tissues. However, when these tumors and the other organs of the host were placed in 9% saline, and dry sodium chloride added slowly, with stirring, the tumors would float before the

TABLE II.
Flotation of Transplantable Mouse Tumors.

Tumor	Conc. of NaCl sol. at 22°C (%)				
	6	7	8	9	10
Crocker sarcoma 180		S*	F*		
Lewis " T241		S	F		
Sarcoma MA387		S	F		
Patti myxosarcoma		S	F		
Mammary adenocarcinoma EO 771	S	F			
Wagner osteogenic sarcoma	S	F			
Ridgway " "	S	F			
Patterson lymphosarcoma	S	F			
Mecca " "	S	F			
Harding-Passey melanoma				S	F
Andervont hepatoma				S	F

* S indicates sink; F indicates float.

TABLE III.
Flotation of Normal and Tumor Tissues (Rat).

Tissue	Conc. of NaCl sol. at 22°C (%)					
	6	7	8	9	10	11
Flexner-Jobling carcinoma	S*	F*				
Walker carcino- carcinoma 256	S	F	F			
Jensen sarcoma		S	F			
Sarcoma R39		S	F			
Heart			S	F		
Liver					S	F
Spleen				S	F	
Kidney			S	F		
Muscle					S	F

* S indicates sink; F indicates float.

other tissues (cf. Sarcoma 180 in Fig. 1). Hence even these relatively heavy tumors were lighter than normal organs in the same mouse. Moreover, the specific gravity of hepatoma, though relatively high, is much less than that of normal liver tissue.

Similar studies in the rat are summarized in Table III. The same observations hold true in this animal, all of the tumors being of lesser density than any of the other organs tested. All determinations of tumors were done on normal-appearing tumor from the periphery, both in the rats and in the mice. However, in addition to normal tumor, areas of Flexner-Jobling carcinoma which were at least 90% necrotic were tested, and yielded the same readings as healthy tumor. Ridgway osteogenic sarcoma in AKm mice yielded a reading of 6 to 7 whether healthy tumor, 50% necrotic areas, or hemorrhagic areas were tested.

Determinations of specific gravity of two types of spontaneous tumors in mice yielded similar information. Spontaneous mammary adenocarcinoma in Rockland albino mice had

a density equivalent to 6 to 7% sodium chloride solution. Spontaneous mammary adenocarcinoma in C₃H mice read 7 to 8%.

In a limited experience with human material, the results seem similar to those in the experimental animal. A single specimen of carcinoma of the rectum obtained at operation read between 7 and 8. A retroperitoneal lipomyxosarcoma removed at autopsy floated in 3.5% sodium chloride solution (this was a grossly fatty tumor). In this case, and 3 cases of lymphosarcoma and one of myeloblastic leukemia examined postmortem, heart and muscle ranged from 7 to 8 or 8 to 9; kidney, 7 to 8 (6 to 7 in lipo-myxosarcoma and myelogenous leukemia); spleen, 8 to 9; liver, 9 to 10; lymphosarcoma read between 6 and 7.

The organs of mice bearing Patterson lymphosarcoma (2 weeks after tumor implantation) were lighter than those of normal AKm mice (heart 8-9; liver 10-11; spleen 8-9; kidney 8-9; muscle 9-10). The liver and spleen were grossly enlarged and infiltrated with tumor in these mice.

Summary. 1. The specific gravity of tumor and normal tissues of mice and rats was determined by flotation in sodium chloride solutions of known concentrations.

2. The density of tumor tissue was always less than that of heart, liver, spleen, kidney, and muscle of the host.

3. Several human tumors were tested and exhibited the same property.

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Effect of Infectious Myxoma Virus on Glycolysis of Chorioallantoic Membrane of Chick Embryo.* (17768)

ERNEST KUN AND MARGARET H. D. SMITH.†

From the Departments of Medicine, Division of Infectious Disease, and Pediatrics, The Tulane University of Louisiana, New Orleans.

Little is known of the effect of viruses on the cell metabolism of the infected host. Most of the information originates from experiments on bacteriophage and from studies of the behavior of viruses in tissue culture. The chorioallantoic membrane of the developing chick embryo appeared to be a suitable model tissue for the study of cell metabolism in the presence of a virus for the following reasons: a) It can be infected readily with a great variety of viruses, and the membrane can be used conveniently for *in vitro* metabolic measurements. b) The development of chemical and morphological changes can be followed at any desired time intervals, thus permitting chronological correlations hitherto difficult to obtain on mammalian tissues.

The present paper summarizes some obser-

vations made on the glycolysis of chorioallantoic membranes of 11-day-old chick embryos which had been inoculated with myxoma virus. The measurement of respiration and glycolysis was carried out 48 hours after inoculation. The experiments reported here exemplify the usefulness of this technic (see point a), while time relationships between chemical and morphological effects (point b) will be reported elsewhere(1). Infectious myxoma virus as an infecting agent appeared particularly suitable for these studies, since this virus causes characteristic lesions on the membrane without killing the embryo itself(2-5). Moreover, the

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