stopping the administration of the folic acid antagonist. It is indeed remarkable that a normal peripheral hematologic picture, including a platelet response, occurs under such therapy. Macrocytic erythrocytes and anisocytosis have been observed(8). A definite explanation of the response cannot be made at present.

Conclusions. In 48 human subjects with acute leukemia treated with folic acid antagonists, there were 9 of the 25 subjects who responded to therapy who had an initial thrombocytopenia. In 8 cases of acute leukemia there was a return of the blood platelets to normal levels and the megakaryocytes became more numerous in the bone marrow. In one other patient the platelets became elevated to slightly less than normal. The rise in platelets was usually followed by an increase in neutrophilic leukocytes. The regeneration of the red cells and hemoglobin then gradually responded. The periods of remission varied from 21 to 240 days.

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Comparative Tissue Explant Requirements of Columbia-SK, C(M) and M.M. Viruses, and of Theiler's Encephalomyelitis Virus.* (17766)

DUDLEY B. SHEAN AND EDWIN W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, School of Medicine, Stanford University, Calif.

Cultivation of the "murine strain of SK poliomyelitis virus"(1,2), here designated Columbia-SK virus(3), has been reported by Sanders and Jungeblut(2). This virus was originally reported as a rodent adapted human strain of poliomyelitis virus (Yale-SK strain), but has recently been established to be a member of the encephalomyocarditis (EMC) group of viruses and to be antigenically unrelated to the original or Yale-SK strain of poliomyelitis virus(4-6). Cultivation of the M.M. virus(7), also a member of the EMC group(4-6), in tissue explants, has been reported by Jungeblut(8). Enright and Schultz(9) first cultivated the Col. SK, M.M. and C (M) viruses in developing eggs. The last named virus stemmed from passage material of the Lansing strain of poliomyelitis virus and was later established by Schultz and White(6) to be antigenically similar to the Col. SK virus. It has been observed to induce typical flaccid paralysis in occasional rhesus monkeys(9) and to be more virulent for cynomolgus monkeys(6). Cultivation of the GD VII strain of Theiler's encephalomyelitis virus in tissue explants has been reported by Parker and Hollender(10), and its cultivation in eggs by Dunham and Parker(11), and Enright and Schultz(9). Cultivation of the FA strain in eggs has been reported by

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TABLE I. Results Obtained from Columbia SK, C(M), and M.M. Viruses, and the GD VII and FA Strains of Theiler's Encephalomyelitis Virus, When Grown on Embryo Tissue Explants of Different Kinds. The dilutions given represent ID_{50} titers; zeros indicate absence of infectivity in 10-1 dilution of the culture fluid in the passages indicated.

Virus	Culture passage	Kinds of explants from mouse embryos									Minced 5-day chick embryo
		Brain	Lung	Small intestines	Heart muscle	Tail	Tongue	Skeletal muscle	Liver	Kidney	Spleen	
Col. SK	5	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-4}	0	0	0	0	0	10^{-5}
	10	,,	,,	,,	,,	10^{-2}						,,
	15	,,	10^{-5}	,,	,,	,,						,,
C(M)	5	10^{-8}	10^{-7}	10^{-7}	10^{-5}	10^{-5}	0	0	0	0	0	10^{-6}
	10	,,	,,	,,	,,	10^{-4}						,,
	15	,,	10^{-6}	,,	10^{-6}	10^{-5}						,,
M.M.	5	10^{-7}	10^{-4}	10^{-4}	10^{-6}	10^{-6}	0	0	0	0	0	10^{-3}
	10	,,	10^{-2}	10^{-3}	10^{-5}	,,						,,
	15	10^{-8}	,,	,,	,,	10^{-4}						,,
GD VII	5	10^{-5}	10^{-1}	10^{-1}	10^{-3}	10^{-5}	0	0	0	0	0	10^{-4}
	10	,,	0	0	10^{-1}	,,						,,
	15	10^{-3}			0	10^{-2}						,,
FA	5	10^{-3}	10^{-2}	0	10^{-2}	0	0	0	0	0	0	10^{-2}
	10	,,	,,		10^{-1}							,,
	15	10^{-2}	0		0							,,

Gard(12), and Riordan and Sâ-Fleitas(13).

The purpose of this investigation was to compare the tissue explant requirements of the above viruses in fluid or suspended cell cultures set up with Simms' solution and ox serum ultrafiltrate as described below.

Materials and methods. The Col. SK and M.M. viruses were obtained from Dr. Claus W. Jungeblut in 1942 and 1946 respectively. The source of the C(M) virus has already been stated. The GD VII strain of Theiler's virus was obtained from Dr. Harold H. Pearson in 1945 and the FA strain from Dr. W. McD. Hammon in 1947. The brains and spinal cords of infected mice were harvested soon after the onset of paralysis. These were pooled, ground to a paste in a mortar and made up to a 10% suspension by weight in 50% Martin's peptone solution (MPS II) (14). This suspension was stored overnight in a refrigerator at 4°C, diluted with MPS II to make a 1% suspension, centrifuged at 2,000 rpm for 15 minutes and filtered through a Chamberland L_3 candle. The ID_{50} of these filtrates and of culture transfers, was determined in the usual manner by inoculating individual 10-fold dilutions, made up with MPS II, into 5 or 6 three- to four-week-old mice, 0.025 cc of each dilution being inoculated intracranially. The ID_{50} titers of these filtrates ranged from 10^{-5} to 10^{-7}.

The tissue explants employed were obtained from 14- to 16-day-old mouse embryos, the pregnant animals being killed by ether anesthesia. Explants of the following tissues and organs were employed: Brain, lung, small intestine, heart muscle, skeletal muscle (from the thigh), tail, tongue, liver, kidney and spleen. These tissues were placed in separate Petri dishes containing glucosol(15) previously warmed to 37°C. Cultures were also set

12. Gard, S., *Acta Med. Scand.* (*Suppl.*), 1943, 143.

13. Riordan and Sâ-Fleitas, M. J., *Science*, 1946, v103, 499; *J. Immunol.*, 1947, v56, 263.

14. Levine, M., and Schoenlein, H. W., A Compilation of Culture Media for the Cultivation of Microorganisms, Baltimore, Williams and Wilkins Co., 1930, p. 308 (medium No. 998).

15. Parker, R. C., Methods of Tissue Culture. New York. Paul H. Hoeber, 1938, p. 36.

up with 5-day-old chick embryo tissue, in which the whole embryo was minced and portions added to the nutrient fluid. The individual tissues employed as explants were minced into approximately one mm pieces with curved scissors. One ounce screw top bottles were used as containers. The fluid medium consisted of 3 cc of Simms'(16) solution and 1.25 cc of ox serum ultrafiltrate(16), the choice of these proportions being based on the results of preliminary tests carried out with Col. SK in the presence of brain explants. To this medium 0.25 cc of minced tissue in glucosol solution, and 0.5 cc of an undiluted Chamberland L_3 filtrate of the virus suspension, were added. The cultures were incubated at 37°C, in an incubator provided with a nearly horizontal slowly rotating plate to improve surface aeration of the cultures. Transfers were made at 3-day intervals. In doing this the cultures were centrifuged for 15 minutes at 2,000 rpm, and 0.5 cc of the supernatant was added to the new medium. At the end of 5 or more serial passages the individual cultures were assayed for virus content. This was done on the fluid portion of the medium only, after the cultures had been centrifuged for 15 minutes at 2,000 rpm to eliminate all or most of the tissue particles. The supernatant was diluted to 10^{-1} with MPS II and this dilution was first tested for viral activity. When this dilution proved active, further dilutions with MPS II were made and tested, the original material being stored in the meantime at −30°C. Culture series which yielded negative results on given explants by the 5th passage were repeated at least once. Virus neutralization tests were carried out at the end of these studies to verify the identity of viruses cultivated.

Observations. The results obtained on different explants with the 5 viruses studied are summarized in Table I.

It will be noted that the Col. SK, C(M) and M.M. viruses grew on explants of certain tissues but not on others, and that the GD VII and FA strains of Theiler's virus were the more exacting in their growth requirements. It will be noted, moreover, that certain differences in explant requirements were observed with reference to the latter 2 strains. The GD VII strain, but not the FA strain, grew on explants of tail tissue.

Inasmuch as liver and spleen failed to support growth of any of these viruses an attempt was made to determine whether these tissues might contain some extractable inhibiting substance. Cultures of Col. SK were therefore set up with embryo mouse brain tissue, but in which an extract of liver or of spleen in Simms' solution was employed in place of regular Simms' solution. The virus grew as well on this medium as on the regularly prepared medium. In another experiment, an extract of embryo mouse brain in Simms' solution was added to cultures of Col. SK, set up with explants of liver or spleen tissue. Explants of these 2 tissues still failed to yield growth of the virus.

Discussion. The observation of special interest, of course, is the well defined selection of explants exhibited by the first 3 viruses (Col. SK, C(M) and M.M.). While certain quantitative differences were observed, some of which may rest on experimental factors, the most striking feature of the results obtained relates to qualitative differences. All 5 of the viruses might be expected to grow on explants of brain, and possibly also on extra-nervous tissue, but it is not apparent why growth should occur on certain extra-nervous tissues and not on others. A well defined difference exists, for example between explants from heart muscle and skeletal muscle, and between lung and liver as supports of growth for the Col. SK and the two related viruses. Inasmuch as skeletal muscle failed to support growth, it is in a measure understandable why tongue also failed to do so, but not clear why tail tissue supported growth and tongue did not. Since explants of skin alone were not included in these studies, it is not possible to say what role epidermis may have played in the multiplication of virus in explants of tail tissue.. Schultz and White(6) have observed that adult mice can be easily infected by members of the EMC group of viruses merely by dipping the clipped tip of the tail into a virus suspension, and have ob-

16. Simms, H., and Sanders, M., *Arch. Path.*, 1942, v33, 619.

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 ID_{50} 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 tumors.

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

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1. Arkin, A. M., and Sugiura, K., *Cancer Research*, in press.

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