ocula, there remained a certain amount of bimodality in the distribution of number of bacteria per monocyte in cultures older than 24 hours. This would suggest that in the present and past(1) studies some unrecognized heterogeneity persisted either in the smooth bacterial populations (in regard to properties influencing interactions with mammalian cells) or in the phagocyte population. Preliminary experiments have indicated that differences in the age of individual monocytes at time of initiation of the cultures may account at least in part for such heterogeneity.

Finally, the observations on monocyte destruction *in vitro* suggest certain future studies in regard to the extent to which similar phenomena *in vivo* might modify the course of disease caused by facultative intracellular bacteria such as *Brucella*. Since it is likely that for these organisms residence and multiplication within phagocytes represents an advantage for the pathogen (*e.g.*, protection from bactericidal blood factors and certain antibiotics), induced monocyte destruction by avirulent R type cells, possibly even by appropriate inert particles or other methods, may contribute to the amelioration of the disease. It is possible that such an approach already may have been employed unwittingly in the case of combined tuberculin and antibiotic treatment(5).

Summary. Studies on the *in vitro* interactions between S and R strains of *Brucella abortus* and monocytes from normal or immune guinea pigs have indicated 1) intracellular multiplication of S bacteria and a lack of, or insignificant extent of, multiplication of R bacteria in normal monocytes, 2) differences in the rate of ingestion of S and R bacteria, 3) differences in the rate of destruction of monocytes by S and R types, 4) differences in the rate of intracellular multiplication among S strains, 5) a significant modification of these events in immune monocytes, and 6) an apparently unique behavior of immunogenic, relatively avirulent strain 19-S bacteria in normal monocytes. The relation of these observations to some problems of host-parasite interactions is discussed. It also has been shown how these observations may provide the basis for a new technic of rapid assessment of the homogeneity or heterogeneity of bacterial populations in regard to properties associated with virulence.

1. Pomales-Lebron, A., Stinebring, W. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 78.

2. Holland, J. J., Pickett, M. J., *ibid.*, 1956, v93, 476.

3. Braun, W., *Bacterial Genetics*, W. B. Saunders Co., Philadelphia, 1953.

4. ———, In Brucellosis. *Symposium, Amer. Assn. Adv. of Sci.*, Washington, 1950.

5. Howells, C. H., Swithinbank, J., *Tubercle* (London), 1957, v38, 1.

Received November 12, 1957. P.S.E.B.M., 1958, v97.

Leukemia. VIII. Leukemogenic Effect of Brain Filtrates After Serial Passage Through Mice.* (23753)

STEVEN O. SCHWARTZ, HAROLD M. SCHOOLMAN, WILMA SPURRIER, AND LEROY YATES
Hematology Department, Hektoen Institute for Medical Research of Cook County Hospital, Chicago

Because of the purported ability of cell-free brain extracts to induce leukemia in mice (1-4), experiments were undertaken to test further the validity of the claim that the filtrates were cell-free. The experiments were so devised that they would give evidence also of the presence of a self-perpetuating agent, and would allow for the comparison of cell-free brain filtrates with tumor homogenates as leukemogenic agents.

The demonstration by Furth(5) that a single cell injected intravenously may produce a lymphoblastoma in mice and the reports of Stasney, Cantarow and Paschkis(6,7)

* Aided by grants from Olivia Sue Dvore, U. S. P. H. S. and Am. Cancer Soc.

that even cell particles could produce tumors made it mandatory to demonstrate that cellular contamination could not be held responsible for the results reported. This was made even more important since the interval between inoculation of the animals and development of the lymphoblastoma is of the same order of magnitude whether cell-free filtrates or whole-cell suspensions are used as inocula(3).

Materials and methods. The AKR mice (high leukemic strain) used in the experiments were obtained from The Jackson Memorial Laboratory. The C3Heb mice (very low leukemic strain) were bred from a pedigreed breeding stock originally obtained from The Jackson Memorial Laboratory. The Swiss mice (low leukemic strain) were obtained from Harlan Small Animal Industry, Cumberland, Ind. Filtrates were prepared by a slight modification of the method previously described(1). The brains were ground with sand in a mortar in an ice water bath. The sand was allowed to settle. The supernatant was then centrifuged at 2,500 rpm for 15 minutes, decanted and passed through a Seitz sterilizing pad whose integrity was tested with a saline suspension of *E. coli*. 250 mg of mouse brain or human brain are used per hundred cubic centimeters of saline solution in preparing the cell-free filtrate, and of this 0.5 cc is used for the intraperitoneal and 0.1 for the intracerebral injection. The experiments were so devised that the original material, whether cell-free filtrate of brain or tumor homogenate, was introduced into a heterologous strain with a low incidence of spontaneous leukemia. In the case of the cell-free filtrates of brain, the material was preferentially introduced intraperitoneally in order to invalidate the possible objection that previously inoculated material was simply reharvested and passed in subsequent passages. Nonetheless some intracerebral inoculations were made for comparative purposes. The experiments with tumor homogenates were uniform in that the material was injected intraperitoneally. In the case of the tumor homogenates, parallel experiments were performed with the use of the cell-free extracts of brains from the same mice that served for the tumor source. The heterologous strain mouse was killed 3 days after it had been inoculated and its brain was used for the preparation of a new filtrate, which in turn was inoculated intraperitoneally into heterologous mouse No. 2. Thereafter the process was repeated until the cell-free extract of the brain of mouse No. 5 was prepared. This filtrate was injected intracerebrally into groups of 20 or 30 mice of the original (homologous) strain. These mice were from 9 to 12 weeks old and served as the test animals. Human brain material was identically treated except that Swiss mice served as the passage, and AKR as the test animals. *Heat inactivated filtrates* and filtrates prepared from nonleukemic mouse and human brain were used for controls and were similarly passed. Two additional control experiments were carried out: In these, tumor cell suspensions of Swiss leukemia were prepared by grinding leukemic lymph nodes and tumor tissue in a ground glass tissue mill with 0.85% solution of sodium chloride; 0.5 cc of a 10^6 cells per cc suspension were injected intraperitoneally into C3Heb mice at the start of the serial passage. At the same time, cell-free filtrates prepared from the brains of the same mice that served as a source of the tumor were passed

TABLE I. Results of Serial Passage of Brain Filtrates.

Original source	Route of passage	Passed through	No. mice with leuk./total inj.
C.F.F. of brain			
Swiss mouse			
Leukemic	I.C.	C3Heb	8/20 (Swiss)
	I.P.		16/20
Nonleukemic	I.C.		0/30
	I.P.		"
AKR mouse			
Leukemic	I.C.	Swiss	19/20 (AKR)
	I.P.		17/20
Nonleukemic	I.C.		0/20
Human			
Leukemic	I.C.	"	19/20 "
	I.P.		"
Nonleukemic	I.C.		0/20
	I.P.		"

C.F.F. = Cell-free filtrate.
I.C. = Intracer; I.P. = Intraper.

TABLE II. Comparison of Leukemogenic Effect of Brain Filtrates and Tumor Cell Suspensions after Serial Passage.

Original source	Route of passage	Passed through	No. of Swiss mice with leuk./total inj.
Leukemic Swiss mouse No. 1			
C.F.F. brain	I.P.	C3Heb	9/20
Tumor-cell susp.	"		0/20
C.F.F. brain, heat inactivated	"		0/30
Leukemic Swiss mouse No. 2			
C.F.F. brain	"	"	8/20
Tumor-cell susp.	"		1/20
C.F.F. brain, heat inactivated	I.C.		0/30

C.F.F. = Cell-free filtrate.
I.C. = Intracer; I.P. = Intraper.

through C3Heb mice. After 5 passages, filtrates from both lines were injected into Swiss mice intracerebrally.

Results. The results of the experiments with the brain filtrates from the various sources are shown in Table I. The results of the experiments in which a comparison was made between cell-free filtrates of brain and tumor-cell suspension from the same animal are shown in Table II.

Discussion. The experiments described demonstrate that contamination by cells or cell fragments is *not* the explanation for the results previously reported. If one assumes 100% recovery of the material which is injected intraperitoneally in each passage, the effect of 5 passages is to dilute the original material to approximately 10^{-13}. The activity of the original filtrate is lost when diluted to 10^{-5}. It is, therefore, necessary to assume that the activity has been replicated.

The experiments in which cell-free brain filtrates and tumor homogenates served as parallel starting substances further underline two points previously emphasized: one, that it is not a cellular or cell fragment contamination that is responsible for the results, since in the case of the tumor homogenate infinitely more cells serve as starting points than could ever be assumed as accidental contaminants, and second, that the active agent is present in the brain in a concentration far greater or a form far more active than that found in tumor tissue or leukemic lymph nodes.

Summary. 1. Cell-free filtrates of leukemic AKR and Swiss mouse and human brains were prepared. 2. The filtrates were serially passed through Swiss mice in the case of AKR mouse and human brains and through C3Heb mice in the case of Swiss mice. 3. After 5 passages leukemogenic activity could be demonstrated by reintroduction of the material into the starting strain in case of the mice, and into AKR mice in case of human brains. 4. No activity or little activity could be demonstrated when leukemic tissues served as sources of the original material. 5. The experiments demonstrate that the activity of the material is not dependent on cells. 6. The dilution resulting from the passages described is such as to compel the assumption that the activity has been replicated.

1. Schwartz, S. O., Schoolman, H. M., Szanto, P. B., *Cancer Res.*, 1956, v16, 559.

2. Schwartz, S. O., Schoolman, H. M., Szanto, P. B., Spurrier, W., *ibid.*, 1957, v17, 218.

3. Schoolman, H. M., Spurrier, W., Schwartz, S. O., Szanto, P. B., *Blood, Hematol.*, 1957, v12, 694.

4. Krischke, W., Graffi, A., Krischke, E., *Naturwissenschafter,* 1957, v44, 400.

5. Furth, J., Kahn, M. C., *Am. J. Cancer,* 1937, v31, 276.

6. Stasney, J., Cantarow, A., Paschkis, K., *Cancer Res.*, 1950, v10, 775.

7. Cantarow, A., *Proc. 2nd Int'l. Cancer Congress* 536, 1952.

Received November 18, 1957. P.S.E.B.M., 1958, v97.