(a) the reversal is then attributed to a stimulation of the left cortex by the injected drug and in the second (b) to the biochemical lesion in the right caudate nucleus which appears to be so severe as to preclude restoration of normal function by the administration of atropine or other drugs. It has been shown (11) that ablation of the right caudate nucleus (with or without destruction of the cerebral cortex) results in circling toward the right. The brain areas involved in the production of the adversive syndrome as well as the mechanisms by which drugs affect this system are at present the subject of a series of investigations in our laboratory.

The usefulness of the present method as a bioassay is demonstrated by the results obtained with the experimental drug WIN 2299. It is shown to be similar to Artane, Panparnit and Lergigan in its effect on forced circling. These drugs play an important role in the treatment of Parkinsonism. Clinical trials with WIN 2299 would seem to be profitable from our present results.

Summary. The intravenous injection of scopolamine, Artane, Panparnit, Lergigan or WIN 2299 into rabbits exhibiting the adversive syndrome evoke a reversal in the direction of the compulsive circling. Of the drugs mentioned, only scopolamine acts synergistically with atropine in causing this reversal. The usefulness of the procedure outlined as a bioassay for atropine-like drugs is discussed and two possible mechanisms by which the drugs studied produce this reversal are mentioned.

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Practicability of Shipping HeLa Cell Suspensions for Use in Color Test For Poliomyelitis Antibodies.* (22035)

Murray M. Lipton and Alex J. Steigman.
Department of Pediatrics, University of Louisville School of Medicine, Ky.

The continuous cultivation *in vitro* of the human cancer cell (HeLa) by Gey and coworkers(1), and the important finding that these cells showed cytopathic effects in the presence of poliomyelitis virus(2), has made possible the use of many tests for poliomyelitis investigations without maintaining a monkey colony. Small laboratories are now better able to make such investigations providing ready access to a good supply of HeLa cells is available. The authors have previously reported their observations on the quantitative titration of 2000 human sera against 3 prototype strains of poliomyelitis virus employing as indicator system color changes of medium in tubes containing suspended HeLa cells(3).

This report concerns the practicability of employing suspensions of HeLa cells shipped to a laboratory for use in carrying out the same method.† The observations were undertaken in order to: (a) learn whether a small

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† Suspensions were obtained from George Washington Carver Fn., Tuskegee Institute, Ala., and may be procured from Dr. R. W. Brown there.

laboratory without special equipment could utilize this technic, (b) circumvent shipment of older cultures with heavy cellular growth since these may fall off during shipment, (c) reduce the cost of shipping numerous bottles, and (d) determine the practicability of shipping suspensions of cells for the initial source of a colony. It was observed by Youngner (4,5) that trypsinized monkey kidney cell suspensions could be shipped at the temperature of melting ice and were usable after a day or more. More recently, Melnick and associates (6), have described experiments in which monkey cell suspensions were prepared in India and flown to the United States.

Method. HeLa cell suspensions were prepared at Tuskegee Institute from young and old cultures which were fed shortly before preparation and shipment of suspensions. Cells were cultured in 250- to 500-ml bottles and dispersed with trypsin according to the method described by Scherer, Syverton, and Gey(*2*). For shipment the dispersed cells, 2-2.5 million per ml, were suspended in a medium of 40% human serum and 60% Hanks' solution. Penicillin and streptomycin were added to respective concentrations of 75 units and 75 μg per ml. The volume shipped was 100 to 125 ml in a glass container whose volume was 125 ml. The container was shipped at room temperature. Those suspensions made up on the afternoon of day 0 were received in our laboratory by noon of the following day (day 1). The suspension was stored at refrigerator temperature (4°C) and used usually on day 2 or day 3. The cell count was checked in a hemacytometer upon receipt of suspension and was within a reasonable range of deviation from the number reported shipped (minus 20%). On removal from the refrigerator the supernatant fluid was removed by suction and the cells washed in 40 ml of medium 199 containing 5% monkey serum and additional glucose(3). The low speed centrifugation for 10 minutes at 500 rpm was followed by removal of the supernate and cell washing for a total of 3 times. Finally, the cells were suspended in medium 199 containing 5% monkey serum with the additional glucose, adjusted so that each ml contained 200000 HeLa cells. Resulting suspension was then used in the colorimetric test(3).

TABLE I. Reproducibility of Serum Antibody Titers. HeLa cell bottle cultures vs. HeLa cell suspensions.

	Type I	Type II	Type III
Titers same*	120	112	103
Titers discrepant			
± 1 tube	50	57	62
± 2 tubes	7	15	14
± 3 or more tubes	2	1	6
Total No.	179	185	185
% reproducible (± 1 tube)	95%	91%	89%

* Highest dilution 1:1024.

Results. The technic outlined above has been employed to determine the antibody titers of 185 sera which have been titrated 7 months previously with cells shipped as bottle cultures, and trypsinized just before use to make suspensions for the test(3). Both tests were performed using a single tube per dilution per virus type with the following serum dilutions: 1:4, 8, 16, 64, 256, 1024. Table I includes the number of agreements and discrepancies which appeared when titers were compared. These titers were obtained in scattered tests, 13 different combinations of tests. Replicate titration results differed by no more than ±1 tube whether employing 2-fold or 4-fold dilution series. Sera which were recorded as agreeing by both methods ranged in titer from less than 1:4 to greater than 1:1024. Sera with titers recorded as greater than 1:1024 were not titrated further. Of 179 sera titrated against Type I virus, 120 were in agreement and 50 differed by ±1 tube (95% reproducible). A similar situation obtains with Type II and Type III antibody titrations with a reproducibility of 91% and 89%, respectively.

Forty-six shipments of cell suspensions were utilized in the titration of 4000 sera against Types I, II, and III poliomyelitis viruses. Control monkey sera of known titer, virus pools of known tissue culture infectivity and human sera of known antibody content‡ have, when titrated by this method, yielded results in close agreement with known titers. Tech-

nically satisfactory titrations have been accomplished using cells sent from a central source and treated according to the method described above.

Summary. It has been found feasible to ship large numbers of suspended HeLa cells packed in a small container. Neutralization titers of 185 sera tested with 2 different types of shipped HeLa cells have yielded reproducible results. Under the conditions described the neutralizing capacity of 4000 serums has been tested against 3 prototype strains of poliomyelitis virus in a color test.

‡ Virus pools were obtained from Connaught Laboratories. Control human and monkey antisera were obtained from Dr. Herbert A. Wenner, University of Kansas.

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Plasma Iodine^{131} Levels as Diagnostic Tests for Lethal X-Radiation Exposure in Rats. (22036)

J. B. STORER* AND H. C. SIMONSON.
Army Medical Research Laboratory, Ft. Knox, Ky.

At present there is no single, simple, diagnostic laboratory test for lethal exposure of man or experimental animals to ionizing radiation. In reviews of clinical diagnosis and treatment of radiation injury, Dunham *et al.*(1) and Cronkite(2) have pointed out that determination of the sequence of occurrence of various signs and symptoms is the principal method for establishing the severity of radiation exposure in man. Of various clinical laboratory tests, the white blood cell count appears to be of most value for prognosis(1,2). Relatively few diagnostic tests have been reported for experimental animals. Rosenthal(3) found an opalescence in the serum of rabbits exposed to lethal doses of x-rays and reported a correlation with lethality. Entenman *et al.* (4) confirmed this finding in rabbits but were unable to demonstrate it in other species. These workers also determined plasma phospholipid phosphorus (PLP) levels in a number of species of experimental animals and concluded that though they might be of value for prognosis of mortality in the dog, the PLP level did not serve as a good indicator of radiation injury "since in all species lethal doses of irradiation (LD_{50} to LD_{100}) are required to produce appreciable changes." Hayes and Hewitt(5) separated the plasma lipids of irradiated rabbits into various "classes" by ultracentrifugation and found an excellent correlation between increases in certain classes and mortality. Their method, however, has the disadvantage of complexity and is probably not adaptable to routine use. The uptake of radioactive iron by red blood cells of irradiated animals is known to be well correlated with radiation dose(6-9). The apparent disadvantage to this method is, that while it is sensitive to relatively low dose ranges, the relationship does not hold well in the lethal ranges since maximum depression occurs at less than lethal levels. Gershon-Cohen and Hermel(10) have recently reinvestigated the very early changes in the white blood cell count of irradiated rats and have concluded that such information may be of value in prognosis.

* Present address: Los Alamos Scientific Laboratory of University of California, Los Alamos, N. M.