blood seems to take a longer time than the lung localizing antibody.

Discussion. For the passive transfer method to give an accurate measure of the localization of antibody in a particular tissue, the localizing antibodies should be made as specific as possible against the target tissue in question. If the lung localizing antibodies are fixed by other tissues as well as the lung itself, the disappearance rate of lung localizing activity from the donor's circulation would not indicate the true rate of fixation by lung. However, it has been shown that the anti-lung antibody once localized in the lung does not go to other organs to any appreciable extent, when eluted and reinjected(10). Therefore, we feel that the data obtained here represent a rather accurate estimate of the localization rate occurring in the lung. Thus, the lung localizing antibody seems to be fixed by lungs very rapidly and the responsible antigens must be in close contact with the circulating blood.

The data are not accurate enough to determine whether the trapping efficiency of lung is greater than that of kidney or liver for their respective antibodies. The more rapid clearance by lung may be due to the greater circulation through the lungs which get the full cardiac output as compared with the other organs which get only part.

Summary. Rate of localization of anti-rat lung antibody in lungs was determined by a passive transfer method. The purified anti-lung antibody was injected into donor rats and the lung localizing activity remaining in the circulation was assayed by injecting the plasmas into groups of recipient rats. The lung localizing antibody was cleared from the circulation very rapidly, indicating that the responsible antigens must be in very close contact with the circulating blood.

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An Improved Method for Rapid Laboratory Diagnosis of Poliomyelitis. (26471)

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A common procedure for laboratory diagnosis of poliomyelitis involves first isolation of the viral agent from a stool specimen followed by serological identification of this agent. Attempts to expedite this procedure by carrying out serological identification directly on virus-containing stool extract have not been entirely successful principally because of the presence of non-specific cytotoxins in such material. Various aspects of this problem have been fully described and discussed(1,2).

It will be shown in this report that by differential centrifugation of infected stool extracts, virus can be concentrated and separated from cytotoxic material. Serological

identification can be performed directly on such viral preparations without intrusion of non-specific cellular reactions. Final results are then, in most instances, available in less than 5 days.

Materials and methods. Cell cultures: HeLa cells and monkey kidney (MK) cells were used. About 50,000 HeLa cells were seeded in tubes (13x100 mm) with 1 ml of medium consisting of 20% horse serum and 0.1% yeast extract in Hanks' solution. After 4-5 days' growth, medium was removed and the cell layer washed with Hanks' solution. MK cells were obtained from a commercial source* as primary tube cultures containing approximately 200,000 cells per tube.

Maintenance medium: For both isolation and identification of the viral agent, medium consisted of 2% chicken serum and 0.5% lactalbumin hydrolysate in Hanks' solution.

Antisera: Rabbits were immunized by repeated injections of live virus.

Preparation of stool specimens: A 20% suspension of the specimen was prepared in Hanks' solution (without bicarbonate) and centrifuged at 2,000 rpm for 10 minutes. Supernate was collected and centrifuged at 8,000 rpm for 20 minutes in the No. 40 rotor of the Model L Spinco centrifuge. Supernate was collected and sufficient gelatin (1% concentration in Hanks' solution) was added to bring the final concentration to 0.1%. As shown by Baron(3), presence of gelatin increases the efficiency of centrifugal sedimentation of small viruses. The specimen was then centrifuged at 38,000 rpm for 2 hours in the No. 40 rotor which was allowed to decelerate without braking. Supernate was decanted and discarded. Sediment was resuspended in 2 ml of maintenance medium and penicillin and streptomycin were added to a final concentration of 100 units each per ml. Three 2 ml suspensions were prepared and pooled for each specimen. This preparation (6 ml) which hereafter will be referred to as stool concentrate, was held at room temperature for 30 minutes.

Simultaneous isolation-identification test: To each of 5 one ml portions of stool concentrate were added respectively one ml of (a) type 1, (b) type 2, (c) type 3 antisera, (d) a pool of the 3 antisera and (e) Hanks' solution. An additional 2 ml of (e) was prepared to be used as inoculum for MK cells. Antisera were diluted 1:20 in Hanks' solution prior to use. Mixtures were held 45 minutes at room temperature, then 0.5 ml was added to each of 3 tube cultures of HeLa cells. In the case of specimen (e), which served as the isolation specimen, 3 tubes of MK cell cultures also were inoculated. Controls for the test were set up as follows: (f) Hanks' solution, (g, h, i) each of the 3 antisera, (j) a known type 1 poliovirus, (k) poliovirus plus known type 1 antiserum. One-half ml of each control was inoculated into each of 3 HeLa culture tubes. It was not essential to include controls (g, h, i) in every test after it had been ascertained that a given batch of antiserum was not toxic for HeLa cells. All cultures were then incubated for 2 hours at 37°C after which the inoculum was removed and replaced by one ml of maintenance medium. Cultures were then incubated at 37°C for final observation.

Occasionally the above routine procedure was modified as follows: Cultures were inoculated with 0.2 ml of each specimen and 0.8 ml of maintenance medium. Cultures were then incubated at 37°C for final observation without removal of inoculum.

A schematic representation of preparation of the stool specimen and protocol for the test are shown in Fig. 1.

Results. Interpretation of possible results of the test: The various possible results of this test are considered in Table I. If stool concentrate contains poliovirus type 1, 2 or 3, the reactions indicated in rows 1, 2, 3, respectively, will be observed. These results are frequently unequivocal in 2 or 3 days and in most instances final within 5 days.

If the result shown in row 4 is observed, incubation is continued for 14 days to detect either atypical poliovirus or other slow-growing enteroviruses. If after prolonged incubation cytopathic changes in isolation cultures are observed, the culture fluids are collected for further studies. If growth occurs only in MK cultures (row 5), virus is collected from

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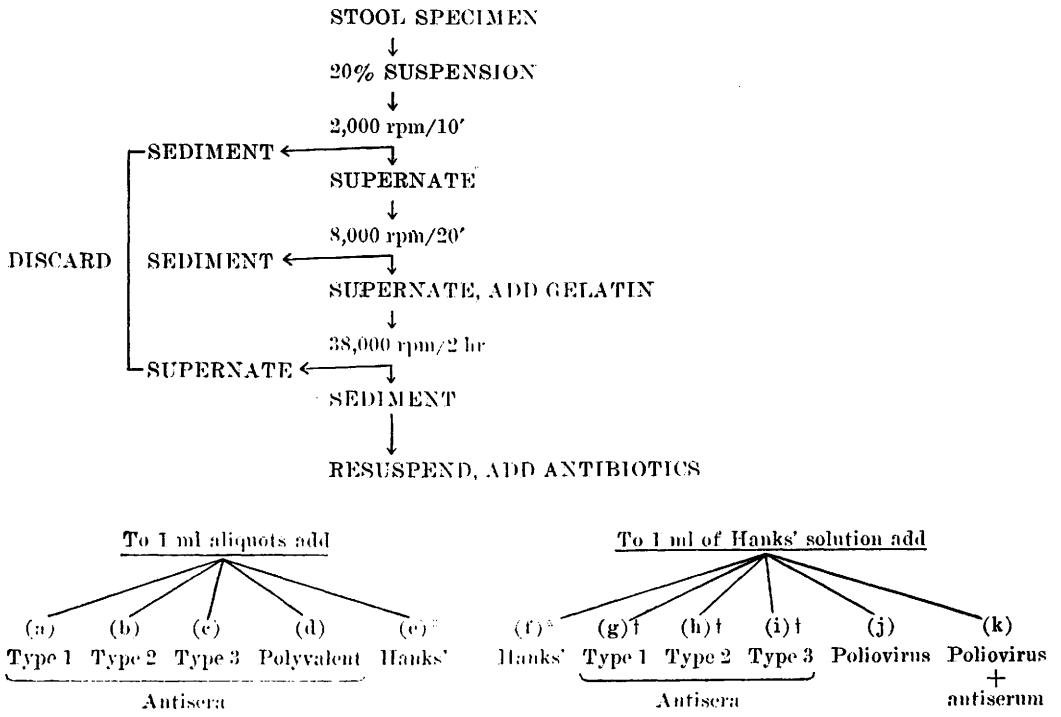

* These are set up in double quantities to allow for inoculation of monkey kidney cultures as well as HeLa cultures.

† These controls are necessary only for untested preparations of sera; subsequently occasional inclusion either as individual or pooled sera is recommended.

FIG. 1. Preparation of stool specimen for use in simultaneous isolation-identification test.

TABLE I. Protocol of Simultaneous Isolation-Identification Test and Consideration of Possible Results.

	Neutralization Stool concentrate + antiserum				*Isolation*	
Row No.	1 (a)	2 (b)	3 (c)	Polyvalent (d)	Stool concentrate + Hanks' (e)	Interpretation of results
1	N*	C*	C	N	C	Poliovirus type 1 present
2	C	N	C	N	C	" " 2 "
3	C	C	N	N	C	" " 3 "
4	N	N	N	N	N—HeLa N—MK	Negative for virus
5	N	N	N	N	N—HeLa C—MK	Virus present that is not poliovirus or is atypical poliovirus
6	C	C	C	C	C	1) Poliovirus but too concentrated for antiserum 2) Poliovirus *and* other virus 3) Virus other than poliovirus
7	C	C	C	N	C	More than one strain of poliovirus

* N = cells appear normal. C = cells show cytopathic effect of virus.

TABLE II. Concentration of Poliovirus in Supernates and Sediments of Stool Suspensions after High Speed Centrifugation.*

Exp.	Specimen No.	Supernate	Sediment
1	794	68×10^5	99×10^6
	870	33×10^5	38×10^7
	896	112×10^5	57×10^6
2	1364	<1	40×10^3
	1148	274	26×10^6
	144	11×10^2	28×10^3
	1305	2	25×10^6
	855	<1	<2
	837	<1	<2
3	F	40	600
	G	20	500
	H	20	580

* Ten ml of stool suspension was centrifuged and sediment resuspended in 2 ml. Titers expressed as plaque forming units/ml.

these cultures and the test is repeated using MK cell cultures.

The results shown in row 6 indicate that either the amount of virus in stool concentrate is in excess of the neutralizing capacity of the antiserum, or both poliovirus and another virus are present, or a viral agent other than poliovirus is present. The test is then repeated with stool concentrate diluted 10^{-3} and 10^{-5}. Finally, if the result shown in row 7 is obtained, it indicates that 2 types of poliovirus are present. The test is repeated with appropriate pairing of poliovirus antisera (*i. e.*, 1 and 2, 1 and 3, 2 and 3).

Control cultures (f, g, h, i, k) should remain normal in appearance for duration of test, while control (j) should show characteristic cytopathic effect of poliovirus. Any other reactions invalidate part or all of the test.

Studies on efficiency of centrifugation: The essential feature of the present procedure that makes the simultaneous test feasible is the partial purification of virus with concomitant elimination of cytotoxic substances. To ascertain that centrifugation was efficient and that the bulk of virus was not being discarded with the high speed supernate, concentration of virus in the supernate and sediment was measured. The assays were performed by plaque technique and results are contained in Table II. In all cases, except for the last 2 specimens of Exp. 2, which contained no detectable virus, concentration of virus in sediment was at least 5-fold greater than in supernate. This is true even for specimens 144, F, G, H which contained comparatively little virus. Concentrations of virus in supernates of specimens 1364, 1148 and 1305 are unexpectedly low in comparison with sediments. It seems unlikely that efficiency of centrifugation is the sole explanation, and other possibilities are under study.

Results of studies on stool specimens: Studies on stool specimens from 176 suspected cases of poliomyelitis were carried out using the consecutive (*i. e.*, isolation followed by identification) and simultaneous procedures in parallel. Of 60 specimens found to contain poliovirus by the simultaneous method, 58 were positive by the consecutive method. Although 18 specimens caused non-specific cytotoxic reactions by the latter method, no such reactions were observed with stool specimens prepared by centrifugation.

With the newer technique, 80% of the positive specimens were typed within 5 days of receipt. The remaining specimens were poliovirus strains which either grew slowly or grew only in MK cell cultures.

The above procedure is equally suitable for isolation and identification of other enteroviruses. In several instances, presence of virus was evident in MK cell cultures but not in HeLa cultures, and these isolates were shown to be Echo viruses. Several Coxsackie viruses were also isolated. Three specimens were found to contain dual agents, one contained types 1 and 3 polioviruses, and 2 contained type 1 poliovirus together with a Coxsackie virus.

Summary. A method is described for simultaneous isolation and identification of poliovirus in stool specimens. The principal feature of the method is partial purification of virus by differential centrifugation of stool extract. Virus so prepared is free of non-specific cytotoxins and is also more concentrated than in the original specimen. Results of studies on 176 stool specimens have shown that the method is rapid, sensitive, and free of complicating non-specific reactions. Positive specimens are typed in most cases within 5 days. The method should also enable more

rapid isolation and identification of other enteroviruses.

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Extracellular Proteins of Staphylococci.* (26472)

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Staphylococci are known to release into the environment in which they grow a number of enzymes and toxins. Knowledge of the origin and function of these extracellular proteins may clarify the manner and degree in which they influence virulence and perhaps communicability. To this end strains of differing pathogenicity were selected with a view to examining the number and distribution of proteins that could be revealed by starch gel electrophoresis, a method capable of giving remarkable resolution as has been demonstrated in studies of proteins of serum (1,2) and other materials(3,4).

Materials and methods. Strains. Strains Mendita and 3895 are examples of classically avirulent coagulase-negative staphylococci of the albus type. Strain Mam-der is regarded as occupying a position intermediate between nonpathogenic and pathogenic staphylococci (5). Strains 4423 and 26925 are examples of *Staphylococcus aureus* phage type 80/81 isolated from the human nasopharynx. Strain 4423 was freely communicable among premature infants in a nursery but was accompanied by an abnormally low lesion rate; strain 26925 was obtained from a healthy person who had no history of recent suppurative lesions. Strains Smith-diffuse and Smith-compact are variants of *Staphylococcus aureus* originally isolated from a case of osteomyelitis. The former is virulent for mice intraperitoneally and is lysed by phage 44A; the latter is avirulent for mice intraperitoneally and apparently is not phage-typeable(6).

Production and Concentration of Extracellular Proteins. The medium used was similar in composition to that of Gladstone and van Heyningen(7) but was modified to exclude substances of high molecular weight that might be present in small amounts in some of the constituents. Two liters of doubly concentrated medium, pH 7.1 - 7.2, were placed in a 6 × 18 in pyrex cylinder fitted with a plexiglass cover containing a large central hole and 2 smaller holes eccentrically located. A large cellophane sac (uninflated width 2¾ in) containing 2000 ml distilled water was suspended from the plexiglass cover by bringing the open end of the sac through the central hole and attaching it to a short length of large-diameter glass tubing. The latter was closed with a rubber stopper carrying 0.7 mm inlet and outlet tubes so that the solution inside the sac could be mixed during incubation by bubbling with gas. The 2 smaller holes carried additional inlet and outlet tubes for mixing of solution outside the sac. The assembly was autoclaved for 30 minutes at 15 lb per square inch, allowed to cool, and placed in a 36° incubator for temperature equilibration.

The inoculum, consisting of saline-washed cocci derived from 2 ml of a 16-hour broth culture, was aseptically introduced into the cellophane sac. Incubation was allowed to proceed for 24 hours during which time the

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