Bacteriological and virological studies were performed on two of these animals. Cultures of liver, spleen, brain, and blood were negative. Stained blood films were negative for blood protozoa.

An agent pathogenic for mice was repeatedly isolated from the lung and spleen of both of these pigs and from one of the brains. It was recovered from the brain, lung, and spleen of inoculated mice and passaged serially by the intracerebral, intraperitoneal, and intranasal routes. On initial passage in 2- and 21-day-old mice the average incubation period was 48 hours and 5 days, respectively. In later passages the incubation period in young adult mice was shortened. Usually adult mice presented flaccid posterior paralysis followed by coma and death, although some acute deaths with no paralysis occurred. Brains from moribund mice contained 10^6 to 10^8 mouse LD_{50} doses. The virus was pathogenic for young hamsters and guinea pigs. It was reisolated and passaged in cell cultures of rhesus monkey kidney, hamster kidney, and HeLa cells. An agglutinin for sheep erythrocytes was demonstrated in infected mouse-brain tissue and in hamster-kidney culture fluid.

The agent was identified by neutralization tests in mice and tissue culture with a hyperimmune rabbit antiserum prepared against the American Type Culture prototype strain of encephalomyocarditis virus (*1*). The identification was confirmed at the Walter Reed Army Institute of Research (*2*). Viruses of the encephalomyocarditis group have never previously been isolated or studied in our Panama laboratories.

A pig, exhibiting fever, listlessness, and anorexia over a 48-hour period, developed a significant rise in neutralizing antibodies to the isolate. Neutralizing antibodies were found in the sera of rats (*Rattus rattus*) trapped on the farm in April 1959. Preliminary studies failed to demonstrate antibodies in the sera of ten men in contact with the swine at the time of the outbreak.

The disease was successfully reproduced by inoculation of two young pigs with the mouse-passaged virus. One pig was inoculated intercerebrally, the other, intraperitoneally. In both animals viremia was demonstrated on the 2nd day, and signs of disease appeared on the 3rd day. The pig inoculated intracerebrally developed progressive paralysis; it was bled and sacrificed on the 11th day. The neutralizing index of this serum was 60. Virus was not recovered from the organs tested. Severe myocarditis and moderate encephalitis were demonstrated histologically. The pig inoculated intraperitoneally became inappetent and listless and died on the 4th day after inoculation. The virus was recovered from the lung and pooled liver-spleen specimens. Histologically severe myocarditis was found. The brain was not examined. The virus strains from both animals were reidentified.

To our knowledge, this report describes for the first time the natural infection of swine with the encephalomyocarditis virus and the most extensive outbreak of encephalomyocarditis infection in man or animals in which the virus was recovered. The outstanding lesion in these naturally infected animals was severe myocarditis. The evidence presented suggests that myocardial failure was the primary cause of death. Contamination of food and water with the excreta of infected rodents and swine possibly contributed to the spread of the virus in this outbreak.

THOMAS G. MURNANE*
U.S. Army Mission to Panama and *Laboratorio de Diagnostico e Investigacion Veterinaria, Panama*
JOHN E. CRAIGHEAD
Middle America Research Unit, Balboa Heights, Canal Zone, and *National Institute of Allergy and Infectious Diseases, Bethesda, Maryland*
HAROLD MONDRAGON
Gorgas Hospital, Canal Zone
ALEXIS SHELOKOV
Middle America Research Unit, Balboa Heights, Canal Zone, and *National Institute of Allergy and Infectious Diseases, Bethesda, Maryland*

Notes

1. The encephalomyocarditis immune serum was supplied by William Pond of the National Institute of Allergy and Infectious Diseases.
2. We thank Malcolm S. Artenstein and Nancy Rogers of the Walter Reed Army Institute of Research for reidentifying the viral isolate.
* Present address: Military Subsistence Testing Laboratory, 1819 W. Pershing Rd., Chicago, Ill.

18 September 1959

Transport of Strontium-90 in Runoff

Abstract. Only a small portion of the strontium-90 that fell on cultivated soils was removed in runoff. The concentration of strontium-90 was usually about 10 times higher in the soil carried by the runoff than in the soil from the plow layer of the plots. Thus, a considerable concentration of Sr^{90} could occur in areas where runoff sediments accumulate.

Strontium-90 is deposited on soil surfaces chiefly through rainfall (*1*). Thus it would seem to be especially likely to move in surface runoff. The extent of this movement was measured on plots which had previously been established for the study of soil losses by rainfall erosion.

Table 1. Strontium-90 in fallout and runoff from corn, oats, and clover plots at La Crosse, Wis., in 1957.

Period	Strontium-90 ($\mu\mu c/ft^2$)			
	Fallout	Runoff from plot		
		Corn	Oats	Clover
3/13–5/14	83	1.1	0.8	None
5/14–5/25	39	0.8	0.6	None
5/25–6/5	30	8.6	8.3	0.4
6/5 –6/15	12	0.2	0.2	0.1
6/15–7/3	20	0.1	0.3	0.2
7/3 –7/16	23	0.1	0.2	0.1
7/16–7/21	12	0.4	0.1	0.1
7/21–8/18	38	0.2	None	None
Total	257	11.5	10.5	0.9

Samples of runoff and rainfall were collected in 1957 at La Crosse, Wis., and Tifton, Ga. (*2*). The La Crosse plots are on Fayette silt loam with a 16-percent slope and are planted to corn, oats, and clover in rotation. The Tifton plots are on Tifton loamy sand with a 3-percent slope and are planted to corn, peanuts, and oats in rotation. One plot was sampled for each crop. The length of the plots was 72.5 ft at La Crosse and 83 ft at Tifton.

Rainfall and runoff samples were collected after each major runoff. The fallout of Sr^{90} was collected by taking rainfall samples in washtubs 3 ft in diameter, which were left in the open near the plot areas at all times. A few quarts of dilute $Sr(NO_3)_2$ solution were kept in the tub to aid in dust retention and to act as a carrier. At La Crosse, the runoff was stirred and an aliquot was taken for analysis. At Tifton, the sediment was dried and mixed, and a sample was taken for analysis. The supernatant was discarded at Tifton because it had a negligible calcium content (*3*)—a finding which indicated that the supernatant would also contain negligible amounts of Sr^{90} since strontium is adsorbed on soils more readily than calcium (*4*).

The Sr^{90} content of the samples was determined at Beltsville, Md. The runoff samples were dried, and 0.1 mole of $Sr(NO_3)_2$ was added as carrier. Strontium-90 was extracted by overnight digestion with hot 4*N* HCl, filtration, and leaching with 1*N* HCl. Interfering radioactive elements were removed by scavenging precipitations; $Y(OH)_3$ was formed in the first of these precipitations, $BaCrO_4$ in the next two and $Y(OH)_3$ in the last. Strontium-90 was determined by separation and by following the decay of its yttrium-90 daughter. Rainfall samples were dried and analyzed by the same procedure, except that no further $Sr(NO_3)_2$ was added and the samples were only slightly acidified with HCl.

The rainfall was a little higher than average during the collection period at both locations. However, it was evenly distributed throughout the period, and there were few severe storms at critical cover periods. For these reasons, soil losses were lower than average. At La Crosse, something over 1 ton/acre was lost on the corn and oats plots and only 0.03 ton/acre on the clover plot. At Tifton, the soil losses were 0.3 ton/acre in plots planted to oats, 0.6 ton/acre in those planted to corn, and 0.7 ton/acre in those planted to peanuts.

The average fallout between runoff collections was 32 $\mu\mu$c of Sr^{90} per square foot at La Crosse and 20 $\mu\mu$c at Tifton. Usually about 1 percent of the fallout appeared in the runoff (Tables 1 and 2). The most notable exception was a runoff at La Crosse on 5 June, during which more than 8 $\mu\mu$c of Sr^{90} per square foot, or more than 25 percent of the fallout, was carried off the plots of corn and oats. Strontium-90 radioactivity is given as of the date of each sample collection.

Only a small percentage of the Sr^{90} that fell on these soils was removed in runoff. The percentage removed was greater on plots with greater amounts of soil loss. Thus, the transport of Sr^{90} would probably increase with more erosive soils, steeper slopes, and cultivation systems which leave bare soil exposed for long periods. However, these conditions are at a minimum in most agricultural areas, and the Sr^{90} content of such soils will not be reduced appreciably by erosion.

Soil samples taken from the plow layer showed 45 $\mu\mu$c of Sr^{90} per kilogram of soil at La Crosse on 11 April, and 16 $\mu\mu$c at Tifton on 11 March, 1957. The concentrations of Sr^{90} were about ten times as high as these in the soil carried by the runoff from most of these plots. However, soil in the runoff from the clover plot at La Crosse, which had the least amount of soil loss, showed 1300 $\mu\mu$c of Sr^{90} per kilogram. Thus it appears that a considerable concentration of Sr^{90} can occur in the limited areas where runoff sediments accumulate.

Table 2. Strontium-90 in fallout and runoff from corn, oats, and peanut plots at Tifton, Ga., in 1957.

Period	Strontium-90 ($\mu\mu$c/ft^2)			
	Fallout	Runoff from plot		
		Corn	Oats	Peanuts
3/11–3/25	10	0.1	0.08	0.2
3/25–4/6	12	0.7	0.04	0.5
4/6–6/4	27	0.3	0.14	0.4
6/4–7/1	26	0.1	0.17	0.1
7/1–7/8	30	0.7	0.21	0.6
7/8–7/28	25	0.1	0.04	0.2
7/28–8/20	10	0.2	0.05	0.1
8/20–10/2	27	0.1	0.04	0.1
10/2–12/2	13	0.2	0.04	0.4
Total	180	2.5	0.81	2.6

RONALD G. MENZEL
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, Maryland

References and Notes

1. S. M. Greenfield, *J. Meteorol.* **14**, 115 (1957).
2. The cooperation of George N. Sparrow and A. Wilson White, Jr., of the Coastal Plain Experiment Station, Tifton, Ga., and of Orville R. Hays and Robert E. Taylor of the Upper Mississippi Valley Conservation Experiment Station, La Crosse, Wis., in collecting the samples is gratefully acknowledged. I am also grateful for the capable assistance of Howard Roberts, Jr., and Douglas R. Keefer in analyzing the samples.
3. R. L. Carter, personal communication.
4. W. R. Heald, in preparation.

5 October 1959

Separation of Serum Antibody Activities by Anion-Exchange Cellulose Chromatography

Abstract. Mumps, *Histoplasma capsulatum.* thyroglobulin, and typhoid H antibodies were found in fraction 1, which contained only gamma globulins with ultracentrifugal sedimentation coefficients of 6.6 Svedberg units (S). Typhoid O antibodies and rheumatoid factor were only in fraction 5, composed principally of gamma macroglobulins with ultracentrifugal sedimentation coefficients of 18 S. In contrast isohemagglutinins, Rh antibodies, and anti-human liver nucleoprotein activities were found in two chromatogram fractions (1 and 5) with different physicochemical properties.

The gamma globulins in normal human serum are composed of two major groups of proteins. Ninety to 95 percent of the total gamma globulins have ultracentrifugal sedimentation coefficients of 6.6 S and 5 to 10 percent are 18-S gamma macroglobulins. The 6.6-S gamma globulins are known also to be a heterogeneous group of protein molecules with differing electrophoretic mobility and hexose content (*1*). Anion-exchange cellulose chromatographic techniques have been used to separate the 18-S gamma macroglobulins from most of the 6.6-S gamma globulins and also to subdivide the 6.6-S gamma globulins into four arbitrary fractions with distinctive physicochemical properties (*1*).

In view of this physicochemical evidence of gamma globulin heterogeneity and of the capacity of anion-exchange cellulose chromatography to fractionate the gamma globulins, a study of the distribution of normal and abnormal physiologic activities among the chromatographic gamma globulin fractions was undertaken.

Sera containing antibodies to viral and bacterial antigens and antibody-like activity against human tissue or serum components were collected. Two or more sera were utilized for each activity tested except in the case of mumps, histoplasmosis, and rheumatoid arthritis sera (*2*).

Fractionation by zone (polyvinyl block) electrophoresis (*1*) of sera from each category except rheumatoid arthritis was performed. The activities in each instance were found among the gamma globulins. The gamma globulin fractions were then pooled, concentrated by ultrafiltration and chromatographed as described below.

Each whole serum (1.0 ml) and electrophoretically prepared gamma globulin (from 1.0 ml of sera) was chromatographed as described in detail elsewhere (*1*) on diethylaminoethyl (DEAE) cellulose (3) columns. Samples and columns were equilibrated with a starting buffer of 0.02*M* phosphate (*p*H 8) prior to sample application. Proteins were eluted from the column in 150 ml of effluent by a progressively increasing ionic strength gradient while *p*H 8 was maintained, and the protein distribution in the effluent fractions was determined by measurement of the optical density at 280 mμ. Subsequently the effluent was divided into 10 or more pools and, if necessary, the pools were concentrated to 1.0 ml by ultrafiltration and dialyzed thoroughly against *p*H 7.6 buffered saline prior to assay.

The distribution of the gamma globulins on chromatography of whole serum is illustrated in Fig. 1 (upper left corner). The distribution of electrophoretically prepared gamma globulin when chromatographed separately is the same. The physicochemical and immunochemical characteristics of gamma globulin fractions 1 to 5 have been described (*1*). Fraction 1 is the first protein peak and is obtained between 5 and 15 percent of the effluent volume. Fraction 2 is obtained between 15 and 25 percent, fraction 3 includes 25 to 45 percent, fraction 4 ranges from 45 to 58 percent, and fraction 5 from 58 to 80 percent.

The distribution of antibody activities after gamma globulin or whole serum chromatography is illustrated in Fig. 1. Antibodies to mumps virus and to *Histoplasma capsulatum* were found in fraction 1 which is composed of 6.6-S gamma globulins. Antibodies to thyroglobulin in the first three sera tested (*4, 5*) were also found in this fraction.