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VIRUS STUDIES ON
DRINKING FOUNTAIN AEROSOLS
AND ITS POTENTIAL HEALTH HAZARDS

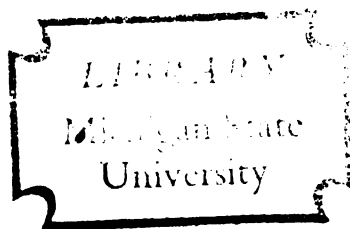
Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Hajime Hayashi
1959

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VIRUS STUDIES ON DRINKING FOUNTAIN AEROSOLS
AND ITS POTENTIAL HEALTH HAZARDS

By
Naajine Hayashi

A THESIS

Submitted to the College of Science and Arts
Michigan State University of Agriculture and
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Appreciation is also extended to Dr. L. C. Ferrisoon and Dr. W. L. Mallison for their helpful suggestions and criticisms of this thesis.

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INTRODUCTION

In an attempt to determine the possible health risks in using public drinking fountains, an angle jet fountain was experimentally contaminated with a virus and studies were made of the resulting water splash and aerosol created.

Water samples were also collected from drinking fountains used by the public and the samples were tested for viral content in tissue cultures.

The design of drinking fountains has changed little since the first public health laws prohibited the common drinking cup in public places in 1909. Berry (1) pointed out that the bubbling fountain was as much a health menace as the common cup on the basis of his experimental demonstration. Dunlap, Hinman, and Maffit (2) reported that both continuous and intermittent flow vertical-jet drinking fountains were insanitary and recommended a slanting-jet fountain with a guard over the orifice. The latter type fountain is widely used today.

Hitchens and Ross (3) found that bacteria remained from 30 minutes to 2 hours on drinking fountain parts that had been contaminated experimentally. They mentioned the possibility of bacterial transfer by splashing water.

Although the study of Anderson, Moss and Gross (4) was not connected directly with drinking fountains, they investigated the resulting aerosol contamination from bacteriological techniques and demonstrated that, when drops

of media containing bacteria fell to a hard surface, the bacteria were disseminated by aerosols.

Bacterial aerosol in connection with drinking fountains was first investigated by Mallmann and Fontes (5). They introduced large numbers of bacterial cells into the jet-stream of a drinking fountain. By exposing agar plates at various distances from the test fountain, they were able to collect viable cells. Mack (6) demonstrated that when small amounts of virus were introduced into a jet-stream of a drinking fountain, a viral aerosol was produced and could be recovered by aerosol sampling methods.

The study of viral agents has been enhanced by Dulbecco and Vogt's (7) tissue culture methods. This method as improved by Younger (8), provided further assistance with cultivation of HeLa cells (Scherer, Syverton and Hey) (9) and other cell lines for viral, biochemical and cytological purposes. It was also found that certain tissue culture lines were more sensitive to some viruses than others (7, 8), making it necessary to use several cell lines for experimental virus work.

With the new techniques for the detection of viruses, it was possible to test the water from drinking fountains used by the public, in an attempt to demonstrate viral agents contaminating the fountains and surrounding area.

The study was divided into two parts: 1. An experimental aerosol study of a drinking fountain in the laboratory under controlled conditions. 2. Attempts to isolate

viral agents from drinking fountains used by public under normal conditions.

1. AN EXPERIMENTAL AEROSOL STUDY OF A DRINKING FOUNTAIN IN THE LABORATORY UNDER CONTROLLED CONDITIONS.

Materials and Methods

The drinking fountain used (Figure 1) in the experimental aerosol work was a jet-angle, standard type, containing an orifice guard. This type of fountain is most commonly found in public buildings. The fountain was installed in the laboratory in a manner simulating usual installation. The virus was introduced into the jet-flow of the fountain by allowing the virus suspension to fall, drop by drop, to the apex of the stream of water in the fountain. No attempt was made to control the flow or pressure of the water and the fountain was permitted to flow throughout the experimental period.

The virus used in the experimental phase of this study was the T-3 bacteriophage. It was produced as lysates from infected Escherichia coli type B cells. The lysates were produced on the surface of synthetic "F" medium (10) by applying the virus to a heavy growth of E. coli cells. After incubation at 37 C for 24 hours, the lysate and debris on the agar surface were flooded with Difco nutrient broth. The resulting suspension was cleared by slow-speed centrifugation and finally filtered through a Millipore*, type HA filter. The virus sus-

*

Millipore Filter Corporation, Watertown 72, Mass.

sensions usually contained 5×10^{10} or more virus particles per ml.

During the experimental period, virus was introduced into the drinking fountain jet-stream, the air about the drinking fountain was sampled by two types of impingers. The most satisfactory impinger was the all glass impinger* (AGI). This impinger has a flow rate of 12.4 to 12.6 liters per minute at or near sonic velocity. Twenty five ml of Difco nutrient broth were used as collecting fluid and contained one drop of Heptadecanol** added as an anti-foam agent.

Assay for virus content of each impinger broth was done by adding 0.3 ml of the impinger broth to 3 tubes (at 45 C) of soft agar and 0.5 ml of a heavy suspension of E. coli cells. The virus-cell mixture was poured over the surface of an agar plate and allowed to solidify. After 12-24 hours incubation at 37 C the plaques were counted to determine virus concentration.

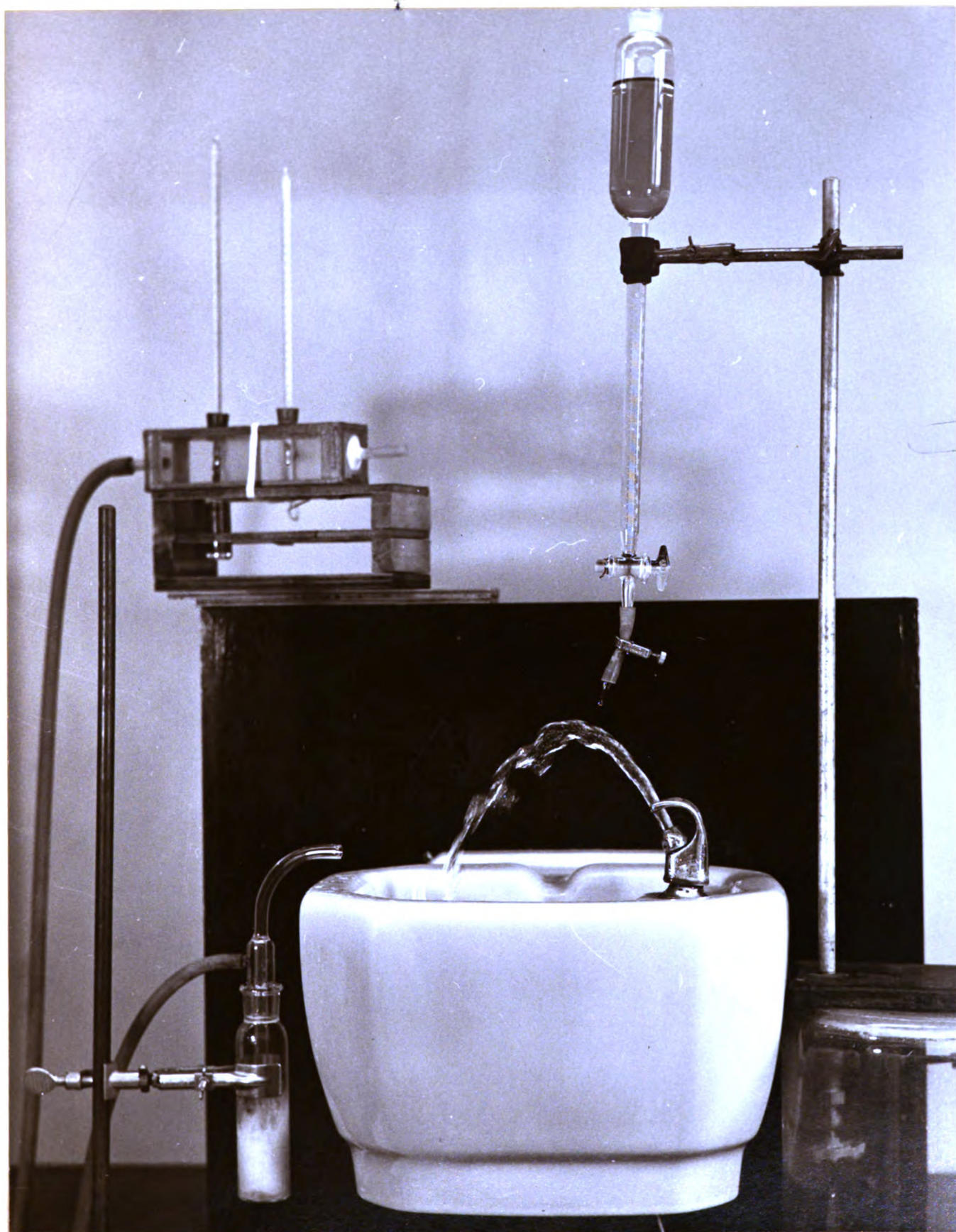
Attempts were also made to use the slit sampler as described by Decker and Wilson (11). The sampler flow was 20.4 liters of air per minute for 1 hour and impingement was made on the surface of agar in large Petri plates previously seeded with young E. coli cells.

* Ace Glass Inc., Vineland, New Jersey.

** Carbide and Carbon Chemicals Company, New York, N. Y.

The relative humidity in the room in which these experiments were conducted was altered to fit the experiments. This was accomplished by atomizing distilled water into the room air, or by boiling water in the room. The relative humidity was determined by drawing the room air through a small orifice in a plastic box containing a wet and dry bulb thermometer.

Figure 1



Results

The photograph (Figure 1) shows the method by which virus was introduced into the jet-stream of the drinking fountain and an AGI collecting the aerosol. At the upper left corner of the picture is the wet and dry bulbs for relative humidity determinations.

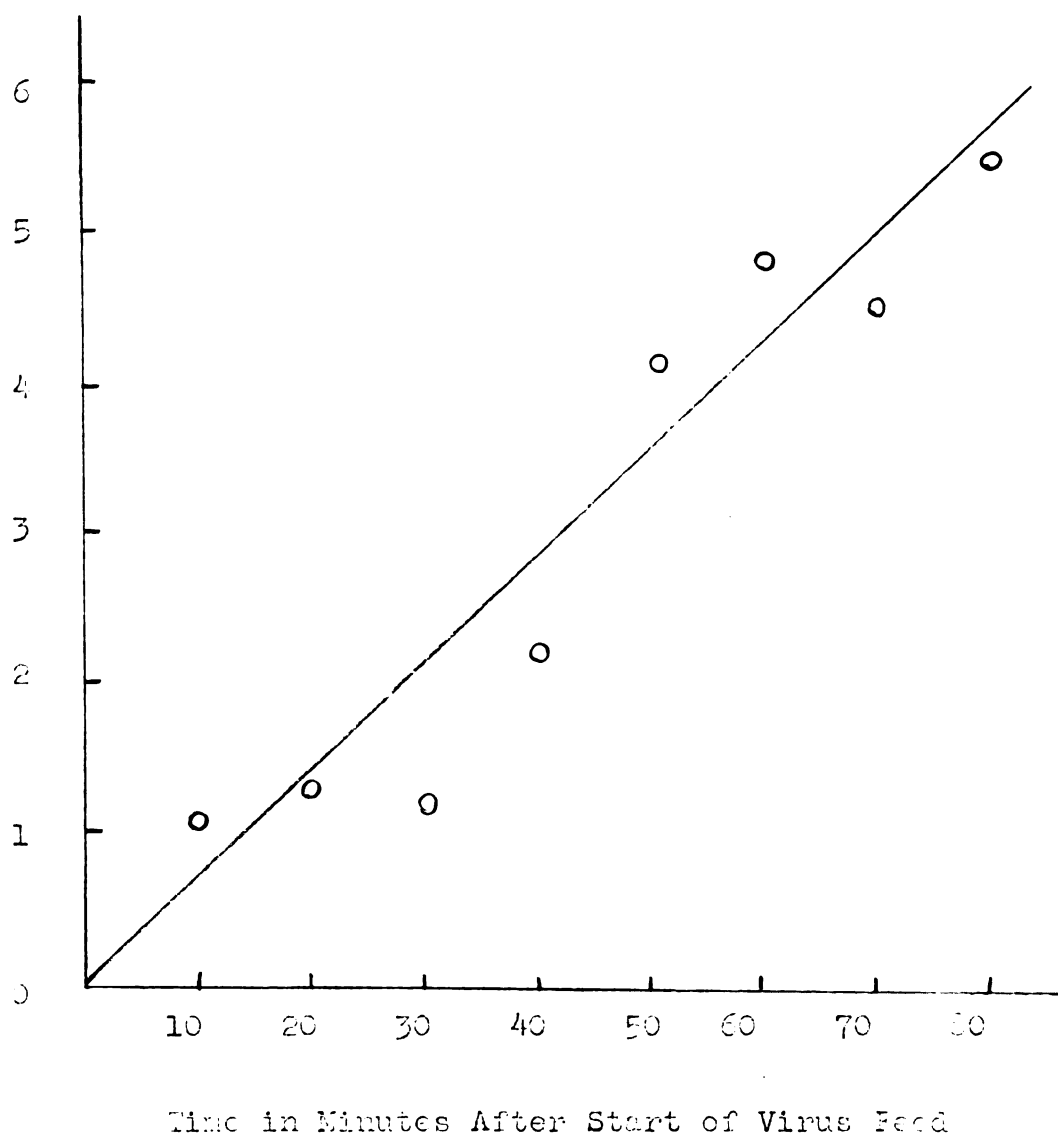
In the first experiment, the object was to determine whether there would be a build-up of the aerosol resulting from continued use of a drinking fountain, therefore, virus was continuously fed into the jet-stream at 4 drops per minute over an 80 minute period. Collection of the aerosol was made 4 inches above the rim of the bowl and down stream from the water flow. Each impinger collected 126 liters of air in 10 minutes and during the test period 8 impingers were employed over the 80 minute period. The results of this test are given in Table I and the data are averages of four tests. The relative humidity during the test period of 80 minutes ranged from 60 to 70 per cent.

As can be seen in Table I there is a gradual rise in virus concentration at the point of collection of aerosol. During the first 30 minutes only one virus particle per liter was collected, but after this period there was a sharp rise of virus concentration to 5 particles per liter. The experiment shows that, the virus concentration in the air around the fountain depends on time and is in proportion to amount of virus introduced into the jet-stream

TABLE I

AEROSOL PRODUCED BY CONTINUOUS INTRODUCTION
OF VIRUS INTO THE JET-STREAM OF A DRINKING FOUNTAIN

Number of
Virus Particles
Per Liter of Air.



of a fountain. Although there is considerable slippage known to exist (unpublished data) with the use of this impinger, the data show that as the amount of virus used increases, so does the collection of virus in the aerosol.

To determine whether the relative humidity was involved in aerosol virus decay, a set of tests were designed in which only the per cent relative humidity was altered. Virus was introduced at 4 drops per minute and samples were collected with the AGI lip placed 8 inches above the bowl and down stream to the water flow. When the relative humidity of the room was adjusted to 32 through 48 per cent, no virus could be recovered in the impingers. When the humidity was increased to 52 per cent, virus was recovered from the atmosphere. At higher relative humidities, increasing amounts of virus were recovered. The data in Table II show uneven recoveries of virus and this will be considered in the discussion.

In the next experiment samples of air were collected at various distances from the fountain. Impingers were allowed to collect 109 liters of air during a 15 minute period. The air in the room was adjusted to 60-70 per cent relative humidity. In Table III the data represent three or more tests for each location about the fountain. The virus suspension was introduced into the fountain jet-stream at 4 drops per minute and sampling begun immediately. The results in Table III show that aerosolized virus can be collected at varying distances from the fountain and

TABLE II
 NOMINAL RECOVERY OF VIRUS IN
 ATMOSPHERE OF INCREASING RELATIVE HUMIDITY

Relative Humidity per cent	Number of particles of virus recovered per liter of air
32	0
37	0
43	0
48	0
52	1.67
57	0.57
62	1.6
62	1.96
62	4.4
69	2.7
71	2.0
71	0.57

TABLE III

ALCOHOL SAMPLING FROM VARYING
DISTANCES FROM THE FOUNTAIN

Impinger Distance in Inches from Apex of Jet-Stream	Location from Jet-Stream	Elevation in Inches from Bowl Rim	Virus Particles Recovered in 100 Liters of Air Tested
0	Apex	6	0.07
6	Upstream	2	0.5
4	Perpendicular	2	3.6
4	Perpendicular	6	0.07
14	Perpendicular	2	4.5
6	Downstream	2	2.00
6	Downstream	6	0.7
18	Downstream	2	0.07

in an area 360 degrees around it. The data also show that the greatest recoveries of aerosolized virus (also see Figure 2 for splash) were down stream and/or perpendicular to the water flow. Virus was found, however, to be disseminated up stream. The largest amount of virus collected was in an impinger placed 14 inches perpendicular to the water flow and two inches above the rim of the bowl. In this case 4.5 virus particles per liter of air tested were collected. The results with the aerosol study agree with the information presented in the following water-splash studies.

Aerosol particles can be made up of a variety of size, shape and composition. In this study the particle is composed of various numbers of virus particles within a spherical water droplet also of varying sizes. At the air flow used, the AEI is capable of sampling the surrounding atmosphere for any aerosol produced by the jet flow and splashing of the water upon contact with the bowl. Those smaller aerosol particles are influenced by the air flow of the impinger and the majority of the smaller aerosol particles are impinged in the collecting fluid. However, many of the water particles, containing various number of virus particles are too large to be influenced by the impinger air flow. These larger droplets of water may, or may not, contain large amounts of virus. It was necessary to determine how far these particles, in the form of splash drops extended away from the drinking fountain bowl.

Figure 2

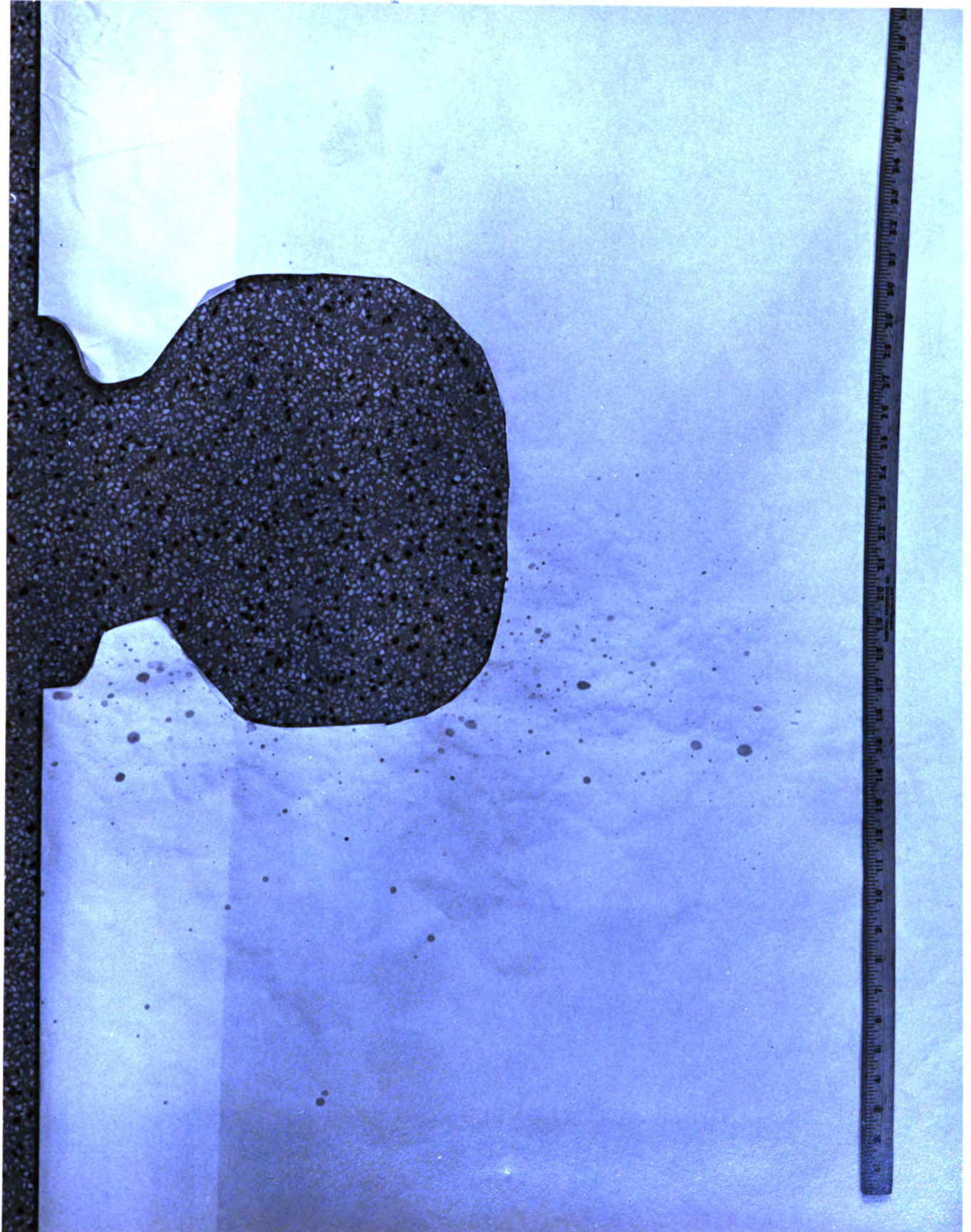
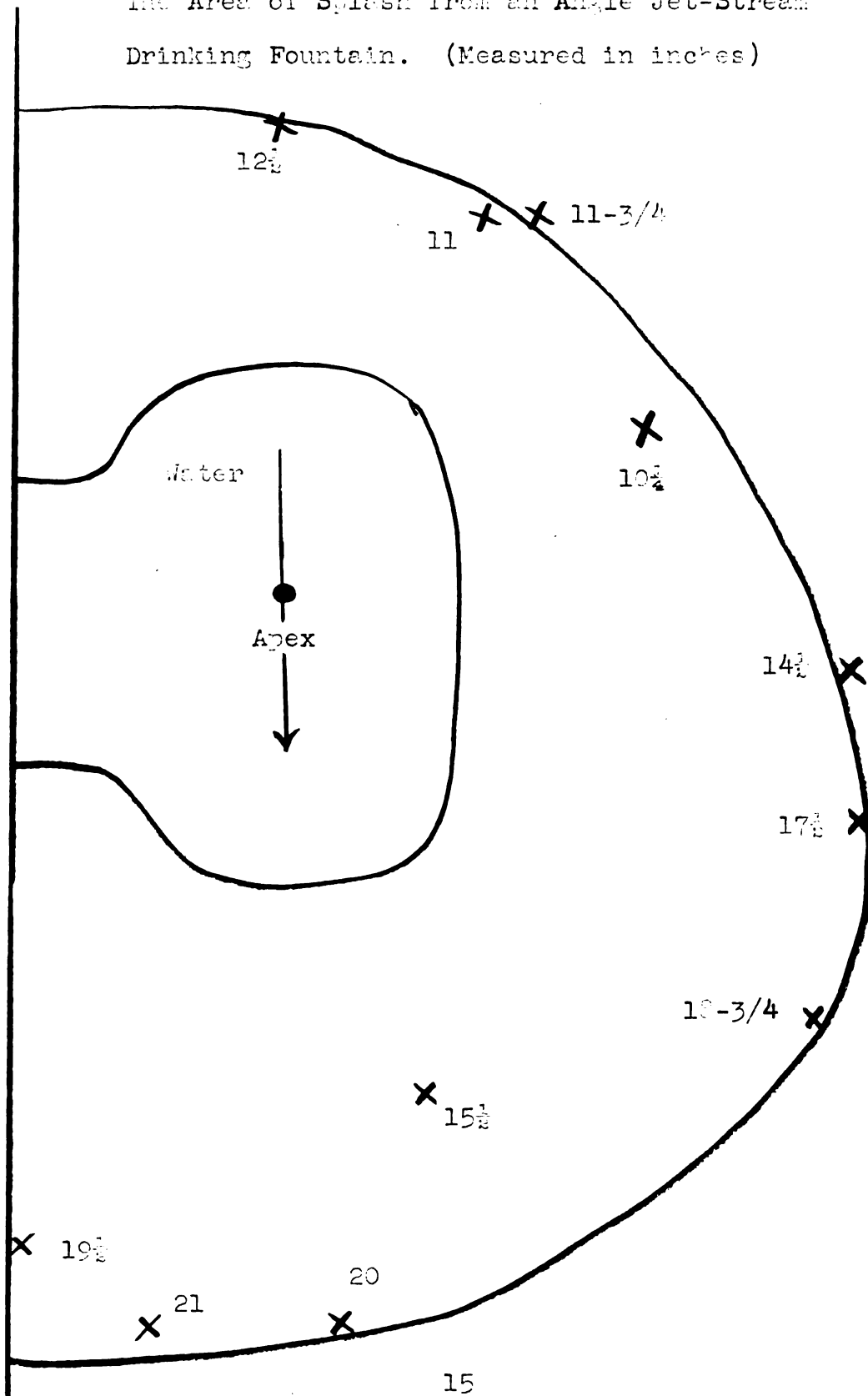


Figure 3

The Area of Splash from an Angle Jet-Stream
Drinking Fountain. (Measured in inches)



In order to visualize the amount and distance these larger particles traveled, the virus suspension was replaced with safranin dye. A large sheet of absorbent paper was placed in the same plane as the outer lip of the fountain bowl after cutting out the shape of the bowl. The fountain water was started and the dye allowed to drip into the jet-stream. The test period was 30 minutes.

The results of this experiment are shown in Figure 2. As was expected, large drops of water, some $\frac{1}{2}$ inch in diameter as represented by splash pattern on the paper, were found a considerable distance from the fountain. The distances these drops traveled from the apex of the jet-stream are given in Figure 3. The measurements were distances in inches radiating from the apex of jet-stream and to the most distant visible dye mark. As can be seen in Figure 3 the large drops were found 21 inches down stream, 12 $\frac{1}{2}$ inches up stream and from 14 to 19 inches perpendicular to the water flow. Anyone using the fountain and passer-bys would certainly have their clothing contaminated with the splash.

The AGI, as used in the above experiments, do not detect viable virus particles when less than 75 particles are impinged in 25 ml of broth. The procedure for assay of this virus uses 0.3 ml of the impinger broth. Each 0.3 ml of broth should contain one virus particle and in assay shows a single plaque formation. If less than 75 virus particles were present in the impinger broth, the

2.3 ml used for assay, depending upon chance, may or may not show the presence of virus in the sample.

To detect small numbers of this virus particle in the aerosols created by the drinking fountain, a slit sampler* was used. The sampler was placed down stream and at the same elevation as the bowl. A 150 x 20 mm culture dish containing Difco nutrient agar was placed inside the sampler. The agar surface in the dish was previously seeded with young E. coli cells. The culture dish was rotated one r.p.m. by clock works. The rotating dish is so placed in the sampler that air entering the sampler, through a .006 inch slit, impinges upon the agar in the culture dish.

Virus was introduced into the jet-stream at 4 drops per minute for one hour. At the same time the slit sampler was started with 1 cubic foot per minute of air flow. At the end of one hour a second sampler was substituted in the same location as the first sampler. The water flow in the fountain was continued but virus was not allowed to enter the jet-stream. The second sampler was operated for one hour. The humidity of the room was again adjusted to 50-70 per cent. After impingement of the virus upon the agar surface, the culture dishes were

* Kindly loaned for this work by the U. S. Biological Warfare Laboratories, Fort Detrick, Frederick, Maryland.

incubated at 37 C until plaques were visible.

The recoveries made of viable virus particles over a two hour period with the slit samplers are illustrated in Table IV. This is the results of ten such tests.

During the first hour, virus recovery ranged from 9 particles to a concentration so great that the plaques were too numerous to count per 1704 liters of air. This, indicating that recovery of virus was dependent upon the amount of splash and aerosol produced. Although no virus was permitted to enter the jet-stream during the second hour period, the slit sampler continued to collect virus. As the bowl of the fountain was contaminated during the first hour, splashing continued to generate aerosol. In tests 3 and 6 no virus was recovered during the second hour period but the other 8 tests show as much as 480 particles per 1704 liters of air.

TABLE IV
RECOVERY OF VIABLE AIR-BORNE VIRUS
PARTICLES WITH THE SLIT SAMPLER

No. of Aerosol	No. of plaques counted in each 1704 liters of air tested	
	First hour with virus	Second hour without virus
1	31	11
2	TNC*	480
3	128	0
4	76	25
5	9	1
6	10	0
7	85	12
8	TNC*	58
9	TNC*	137
10	52	8
Average		72

* Plaques, too numerous to count.

2. ATTEMPTS TO ISOLATE A VIRUS FROM DRINKING FOUNTAINS USED BY THE PUBLIC.

Materials and Methods

Beginning on September 12 and continuing through October 31, 1950 water samples were collected from drinking fountains located in public buildings. Fountains used by students at the university library, men's gymnasium, student union and the auditorium were tested. One fountain in a grade school used by children from kindergarten through the third grade was also tested.

The fountains in the university buildings were probably "cleaned" once a day with a dirty sponge. The fountain in the grade school was always kept clean and was sanitized at least once each day.

Water samples were collected by using gauze pads as described by Moore(12), and consisted of absorbent cotton placed between two layers of cheesecloth (one by two inches) and sewed together around the periphery. The pads were placed in the bowl of the drinking fountains for 24 hours. Approximately 7 ml of liquid were extracted from the pad and placed in a sterile test tube. The samples were then held in a frozen state at -20 C until used. Just prior to tests for virus, the specimens were thawed and 150 units of penicillin and 150 ug of streptomycin per ml were added to each sample to destroy bacteria present. The specimens were held at 5 C for 24 hours and

then a sample of each specimen was introduced into tissue cultures for virus detection. A sterility control was done in brain heart infusion broth on each specimen. The control culture was placed at 37 C and observed after 72 hours incubation period for signs of bacterial growth.

Mouse Inoculation

Since the Coxsackie virus might be encountered, 0.03 ml of each water sample was injected intraperitoneally into a family of one-day-old suckling Swiss mice. The mice were examined daily for 21 days before discarding as negative.

Tissue Cultures

The line of HeLa cells used was obtained from Tuslegee Institute, Alabama. HeLa cells were grown in 8 oz. prescription bottles, in growth medium consisting of 10 per cent inactivated human serum in Eagle's basal medium (13). Test tube cultures were prepared by trypsinizing the above growth cultures. Nine tenth ml containing 10,000 cells suspended in growth medium was implanted into each round, screw cap, 16 x 125 mm tube. Two or three days after implantation and immediately before inoculation of water samples, the growth medium was replaced by the maintenance medium consisting of Eagle's basal medium with 2 per cent inactivated calf serum.

Monkey kidney cells were purchased from Microbiological Associates, Bethesda, Maryland. Upon arrival the 0.5

ml of growth medium was replaced with 0.9 ml of Mixture 119, developed by Morgan et al. (14), containing two per cent inactivated calf serum. The monkey kidney cells were held 24 hours at 37 C before inoculation with water samples.

All media contained penicillin, 100 units per ml, and streptomycin, 100 ug per ml.

Each of three tubes of HeLa cells was inoculated with 0.1 ml of the processed water specimen. The cells were observed every other day for sign of cytopathogenic effect (CPE), over 21 day period.

The water samples which showed evidence of CPE on HeLa cells were inoculated into each of three tubes of monkey kidney cells in the same manner as HeLa cells. They were also observed for 21 days after inoculation.

Results

The number of water samples tested from each drinking fountain from various buildings is shown in Table V. Eighty six samples were tested, 43 samples from the library, 22 from the grade school, 5 each from the men's gymnasium and the student union and 3 from the university auditorium. Of the 22 samples tested from the grade school, 6 samples (27.2%) produced some CPE in the original HeLa tissue culture and additional sample was questionable. A subculture, into the same cell line, with nutrient fluid from the original culture produced some CPE with 5 of the 7 original samples. With an additional subculture (second passage) only 2 of the samples continued to show CPE.

There was a total of 43 samples of water tested from the university library and 7 samples (16%) showed some type of CPE and 3 were questionable. On subculture, again 7 specimens produced some changes in the tissue culture cells to warrant an additional subculture. However, on this third culture (second passage) only one sample affected the cells of the tissue cultures.

From the next two locations (Table V) 18 samples produced only 6 showing CPE of HeLa cells. Additional subcultures of these 6 samples failed to show any effect upon the tissue cultures. The samples from the auditorium building showed 2 samples with CPE but upon passage lost this ability.

TABLE V

RESULTS OF TESTING WATER SAMPLES IN TISSUE CULTURE

No. of samples tested	Hole cells				Monkey kidney cells	
	No. of samples showing CPE*				No. of samples tested	No. of samples showing CPE*
	Orig. pass.	1st pass	2nd pass	3rd pass		
Grade School 22	6 (1)**	5	2	0	7	2
Library 1st Floor 21	3 (1)	3	0	0	4	0
Library Basement 22	4 (2)	4	1	0	6	1
Student Union 9	2	0	0	0	2	0
Men's Gymnasium 9	4 (1)	0	0	0	5	0
Auditorium 3	2	2	1	0	2	0
Total 86	21 (5)	14	4	0	26	3

* CPE - Cytopathic effect

** No. in () - Samples tested with questionable results.

All samples showing any CPE and any questionable samples were transferred to a third tissue passage in HeLa cells. None produced any definite effect upon the cells.

The CPE, as described in detail below, was not unlike that described by Enders (15) on the isolation of measles virus in human kidney tissue culture. If the agent in the water samples was measles, it would be advantageous to test the suspected samples in monkey kidney tissue culture. When the 21 samples that produced CPE and the 5 questionable samples were tested in monkey kidney epithelium, only 2 samples from the grade school and one sample from the library basement produced some CPE. The three samples producing CPE in the original monkey kidney tissue cultures did not show any CPE upon subculturing.

The characteristics of the observed CPE requires some explanation. Figure 4 shows the normal sheet of the HeLa tissue culture cells. When the water samples were placed in contact with the tissue cells, the overall cell sheet was not affected. The first visual change occurred usually on the 2nd through the 4th day after inoculation. The affected area was small and limited to a few places where the cell began to show "rounding" and eventually became more transparent than the neighboring cells. Giant cells were frequently formed in this area and appeared quite granular. Continued incubation of these cultures showed that the affected area increased with time, the rounded

cells were partially detached from the glass and sometimes, but not always, the spindle-like cells would replace this area. Figure 5 illustrates the transparent, affected cells and giant cell formation. In Figure 6 the majority of the round cells have been removed and the openings in the cell sheet shows debris and long processes connecting the cells.

Continued observations did not reveal any spread of the affected area and the remaining cells developed granulation and degenerated similar to those in control cultures as the cultures became older.

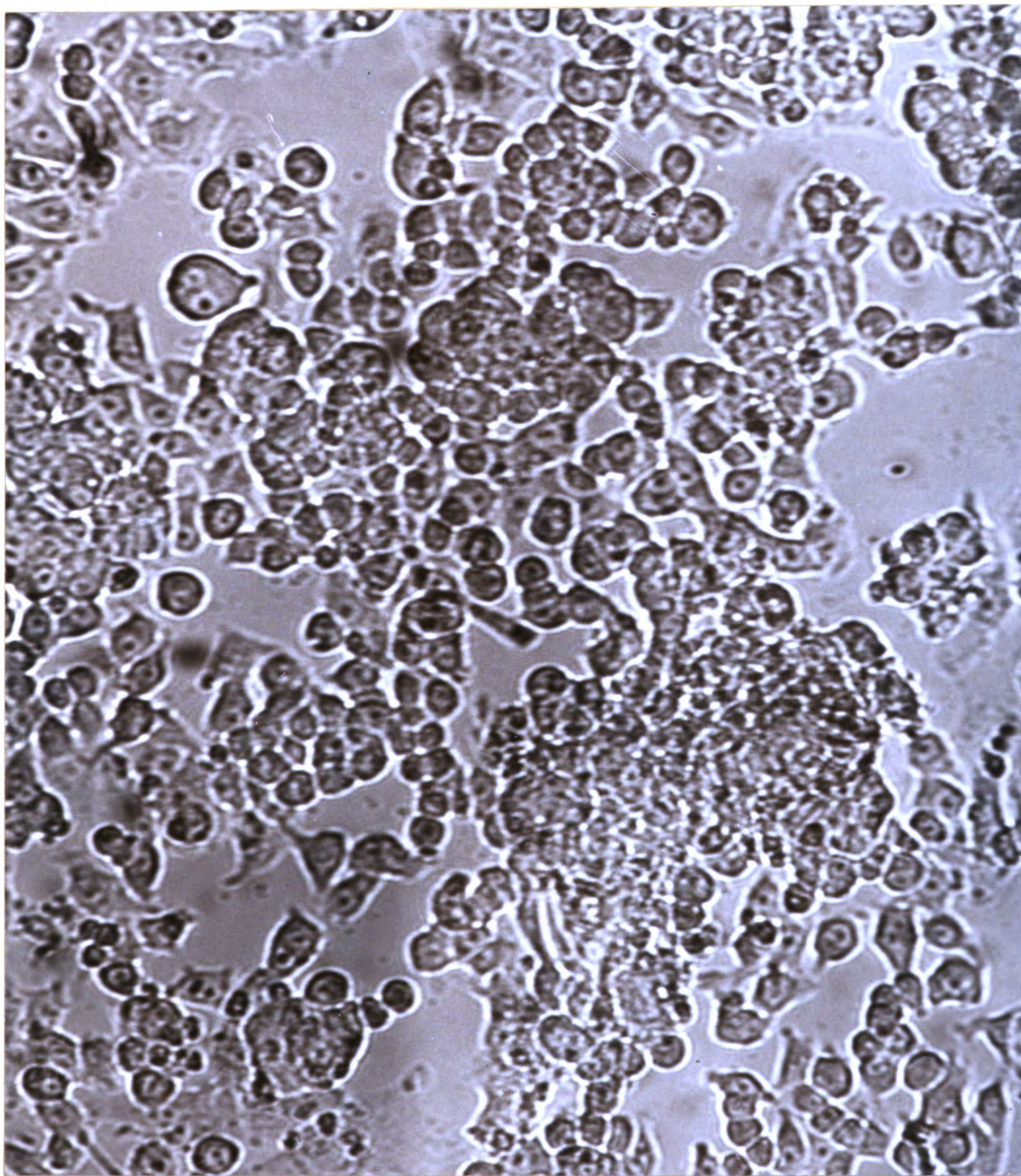
Two types of CPE were observed in the monkey kidney cells inoculated with water samples from the drinking fountain. Only those samples showing some CPE in HeLa cells were inoculated into monkey kidney tissue cultures. For comparison, Figure 7 shows the normal cell sheet of monkey kidney epithelium. Water samples from drinking fountains produced besides that already described for the HeLa cells, a cloudy reaction or lace-like network in the monkey kidney cultures. Figure 8 shows the beginning of the giant cell formation and this is continued in Figure 9 where the large vacuoles are beginning to form. The final stage of this process is illustrated in Figure 10 where the cells were for the most part replaced by the lace-like network.

To examine the individual cells of the tissue culture, a slight modification of the method described by Enders (15) was employed. The cells were stained with hematoxylin and eosin stain and the cells examined for any changes in cell

morphology. In Figure 11 the cells of normal monkey kidney cells illustrate a compact cell sheet with deeply staining nuclei and small less deeply stained cytoplasm. The normal cells contain one or more nucleolus which is visible in the majority of cells. Figures 12 and 13 were taken of the cells in those areas where the cells were in the process of being destroyed. In Figure 12 the nucleus is reduced in size and the cytoplasm has increased in amount. The nucleolus has become indistinct in many of the cells. The formation of a giant cell is seen in Figure 13 and cytoplasm of many of the cells are becoming indistinguishable from the back ground. A large vacuole is visible in the upper right corner of Figure 14. These vacuoles are what produce the lace-like arrangements as seen under lower magnification. On the left side of Figure 14 many degenerated cells are to be seen.

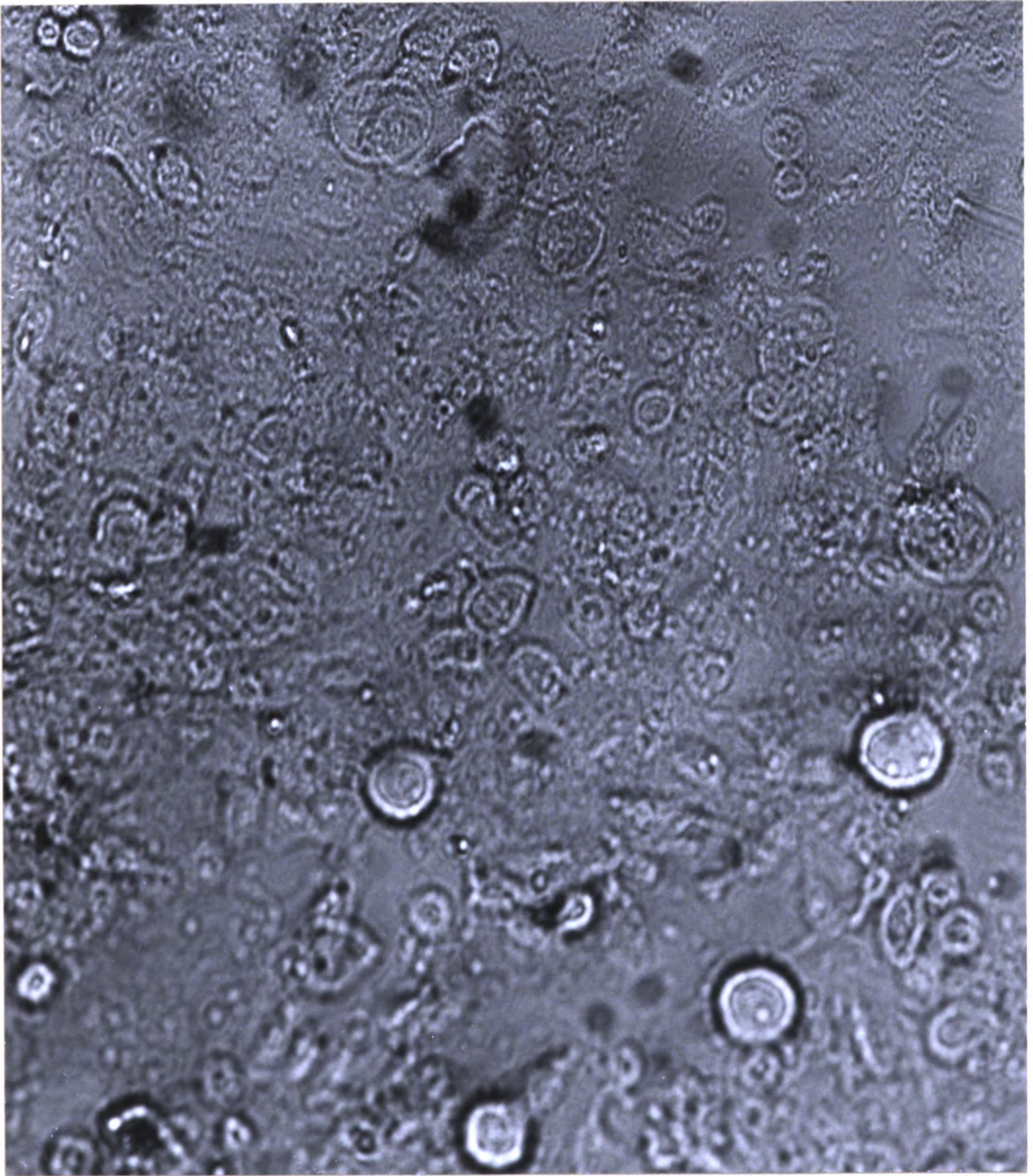
A sample of each of the 36 water specimens was injected into a family of one-day-old Swiss mice. None of the samples produced disease in the mice during a 21 day observation period.

Figure 4



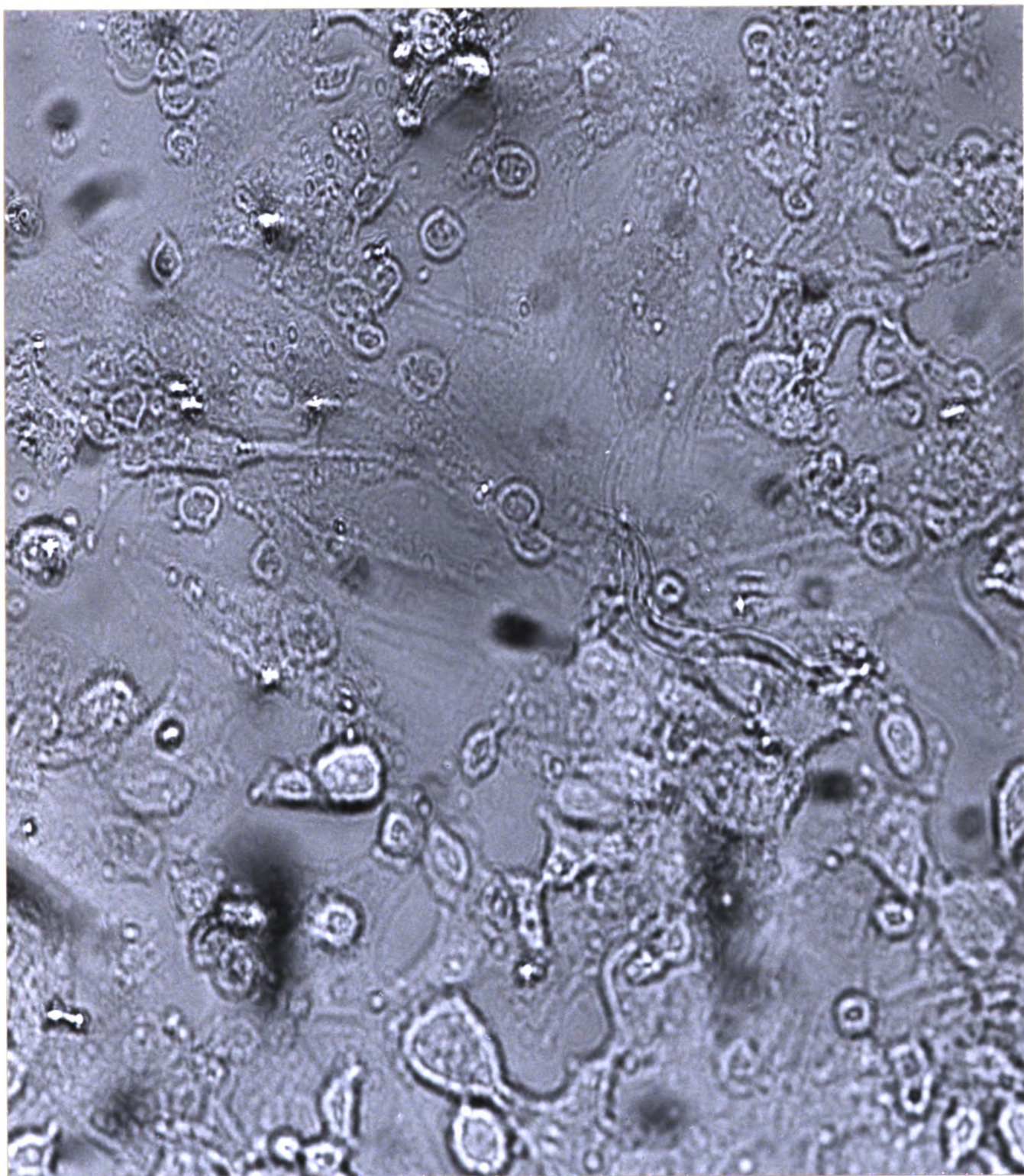
Outgrowth of normal HeLa cells in an uninoculated tube culture. Control for Fig. 5 and 6. (600x)

Figure 5



Appearance of giant cells in culture, 5 days after inoculation. (810x)

Figure 6



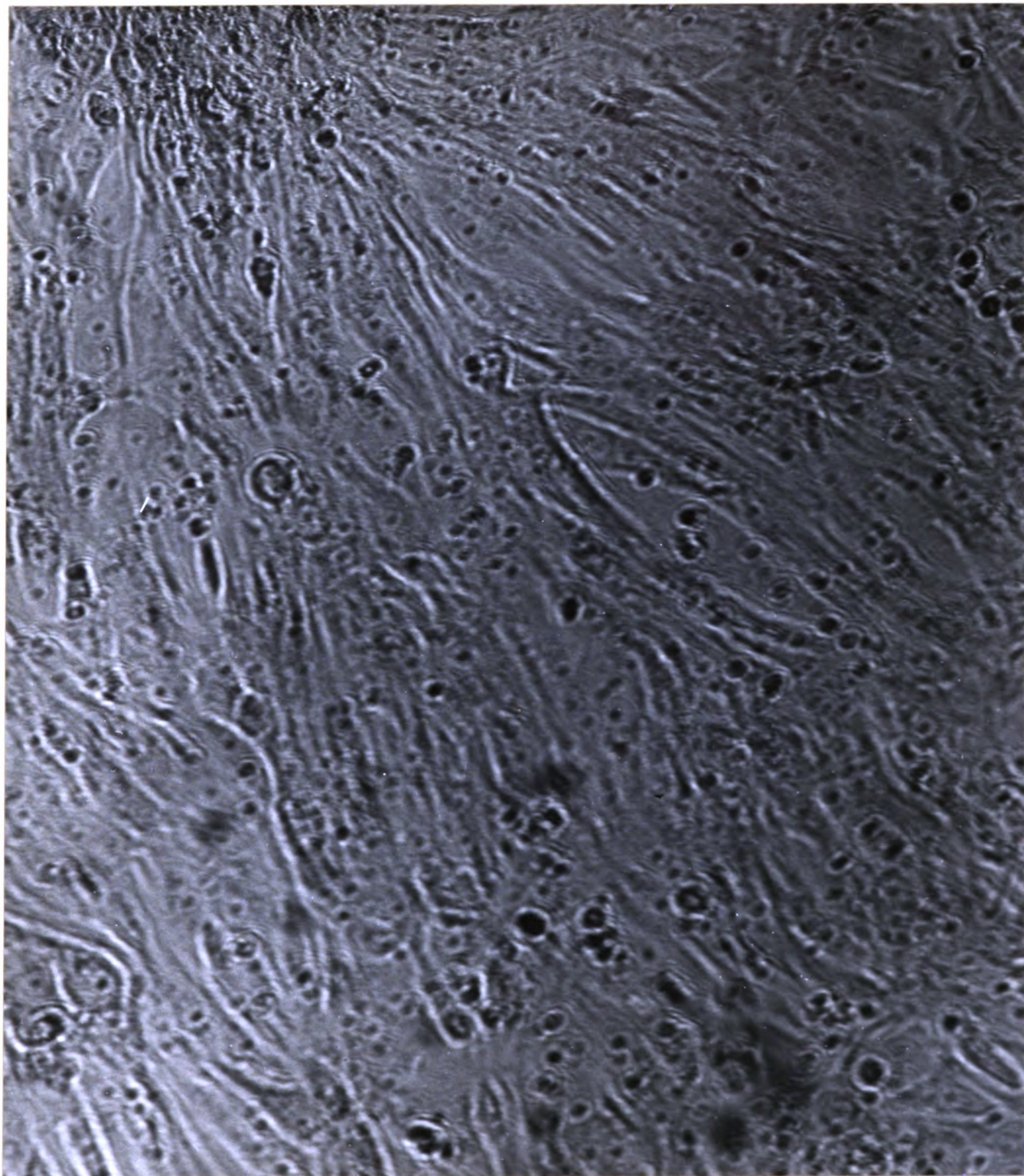
Cell aggregation and granulation, 8 days after inoculation. (810x)

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Figure 7



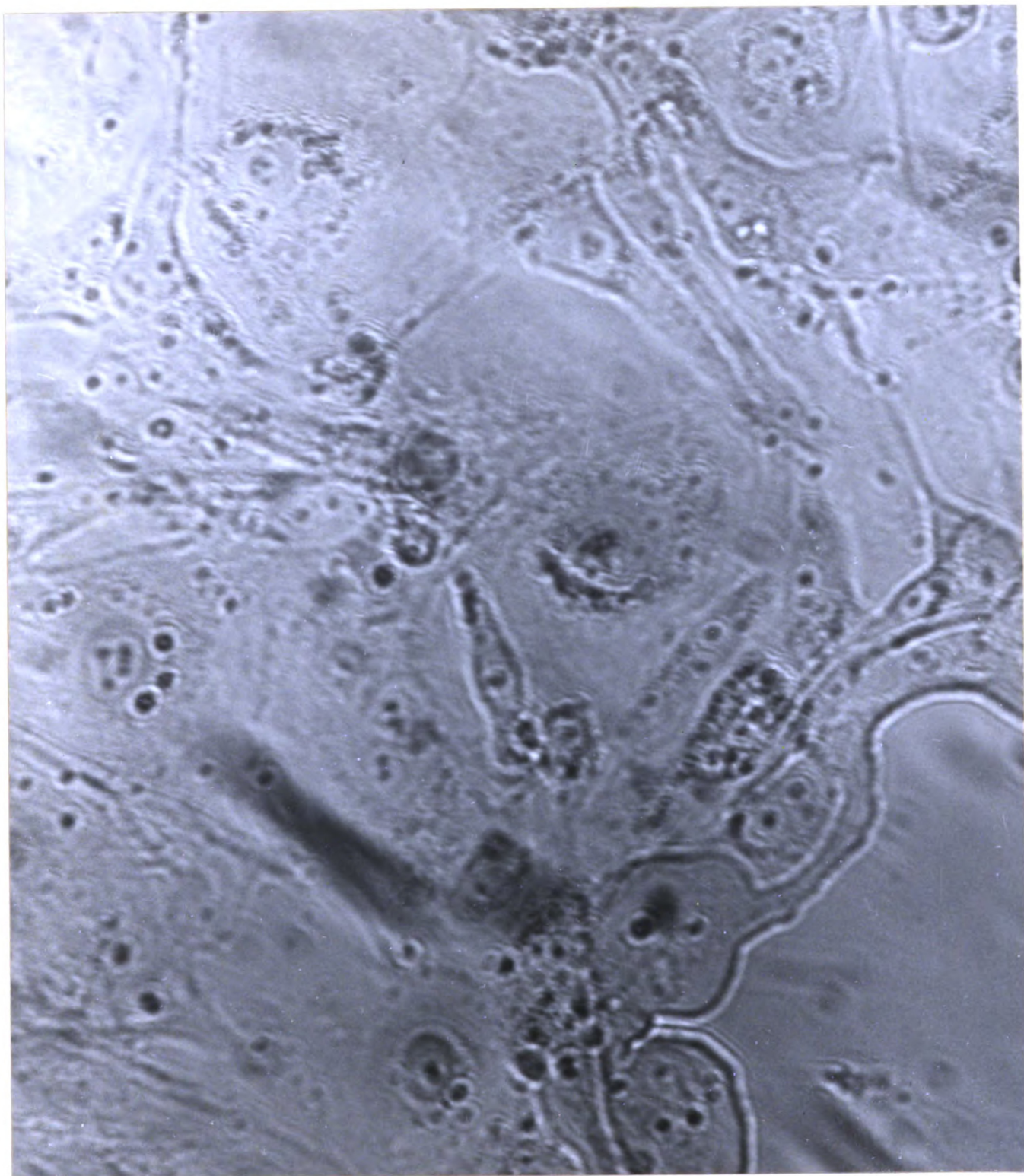
Outgrowth of normal monkey kidney cells. Control for
Fig. 8, 9 and 10. (600x)

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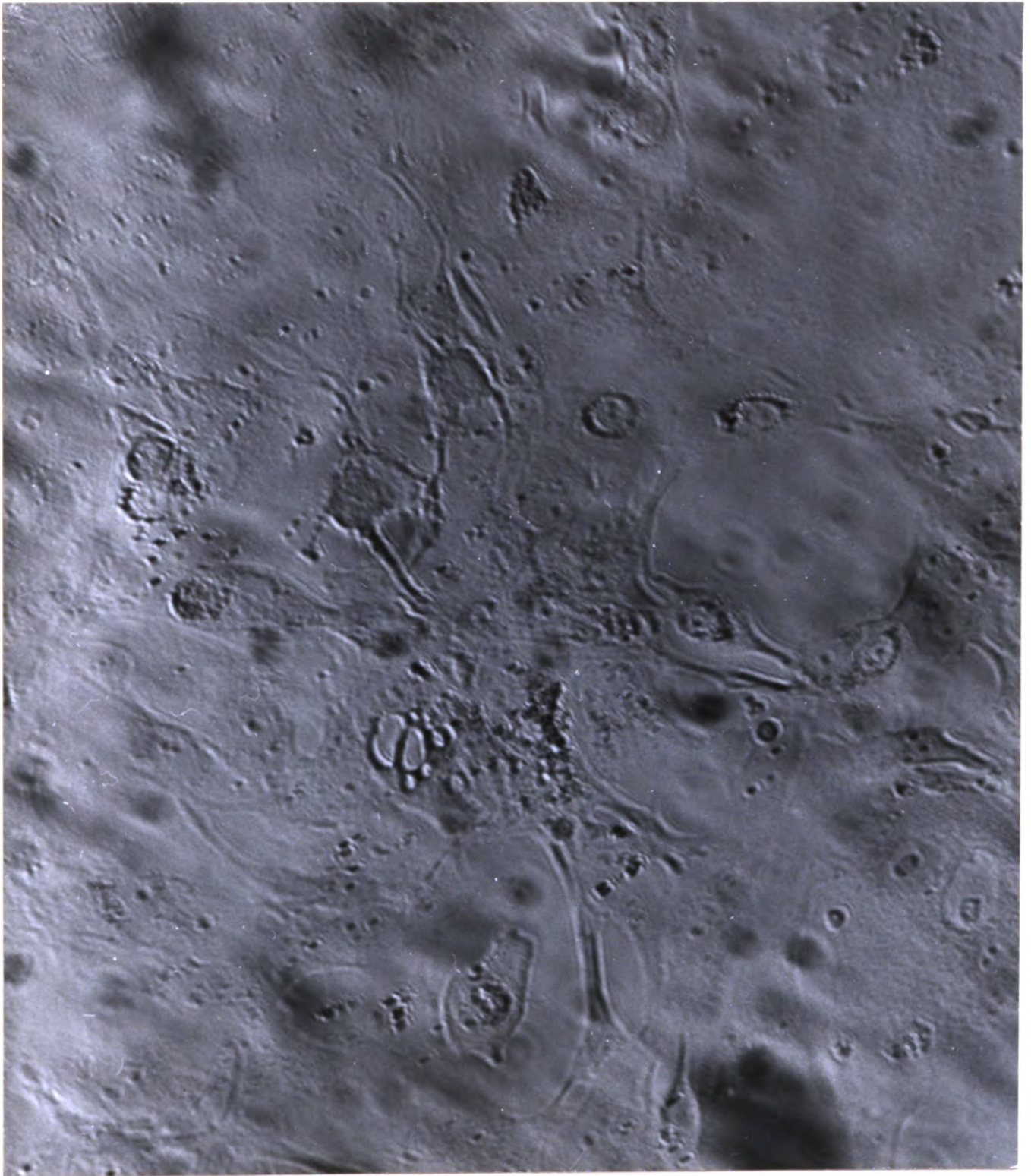
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Figure 8



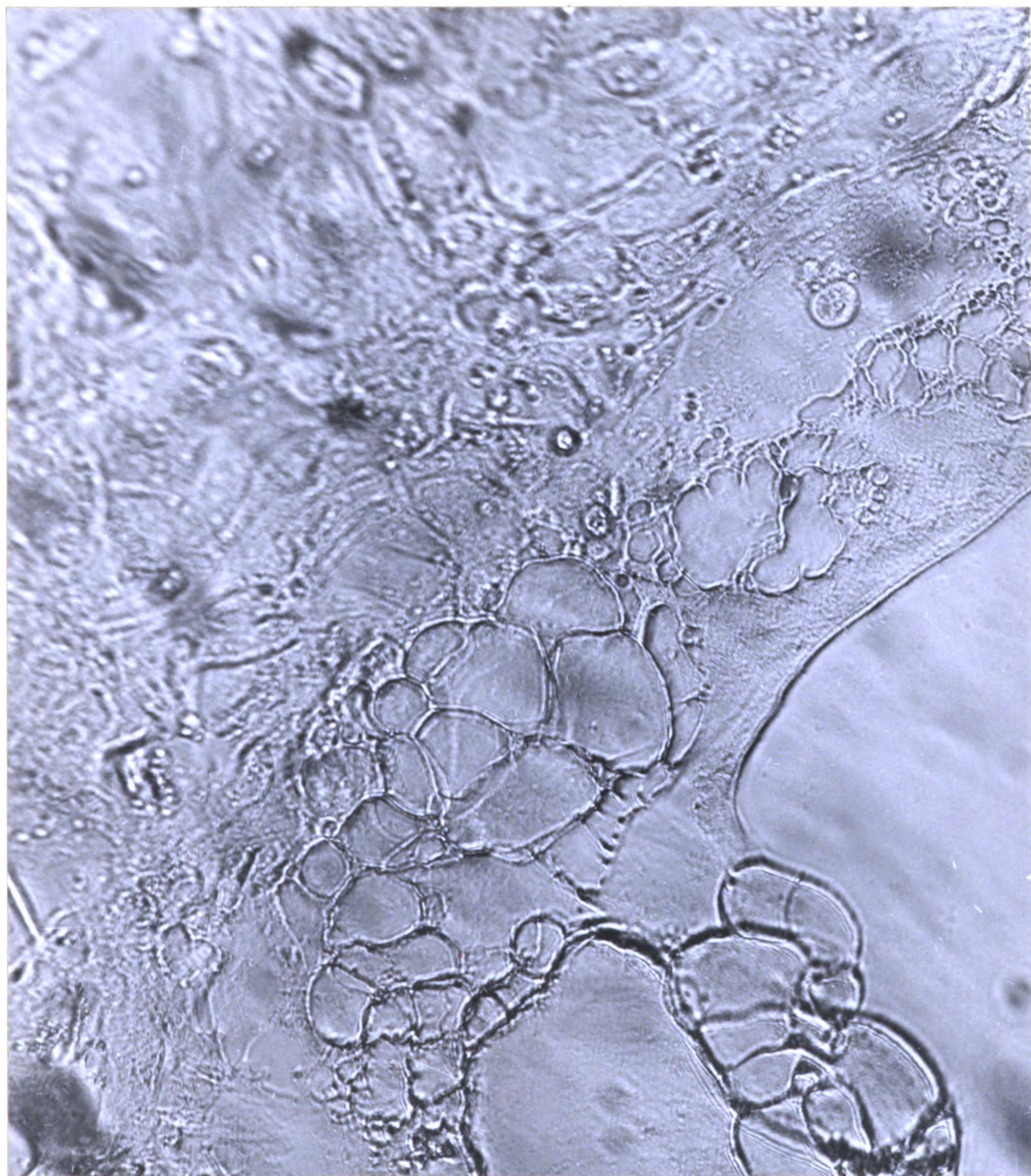
Giant cell formation with multinuclei, 7 days after inoculation. (600x)

Figure 9



Giant cells with small cytoplasmic vacuoles, 7 days after inoculation. (600x)

Figure 10



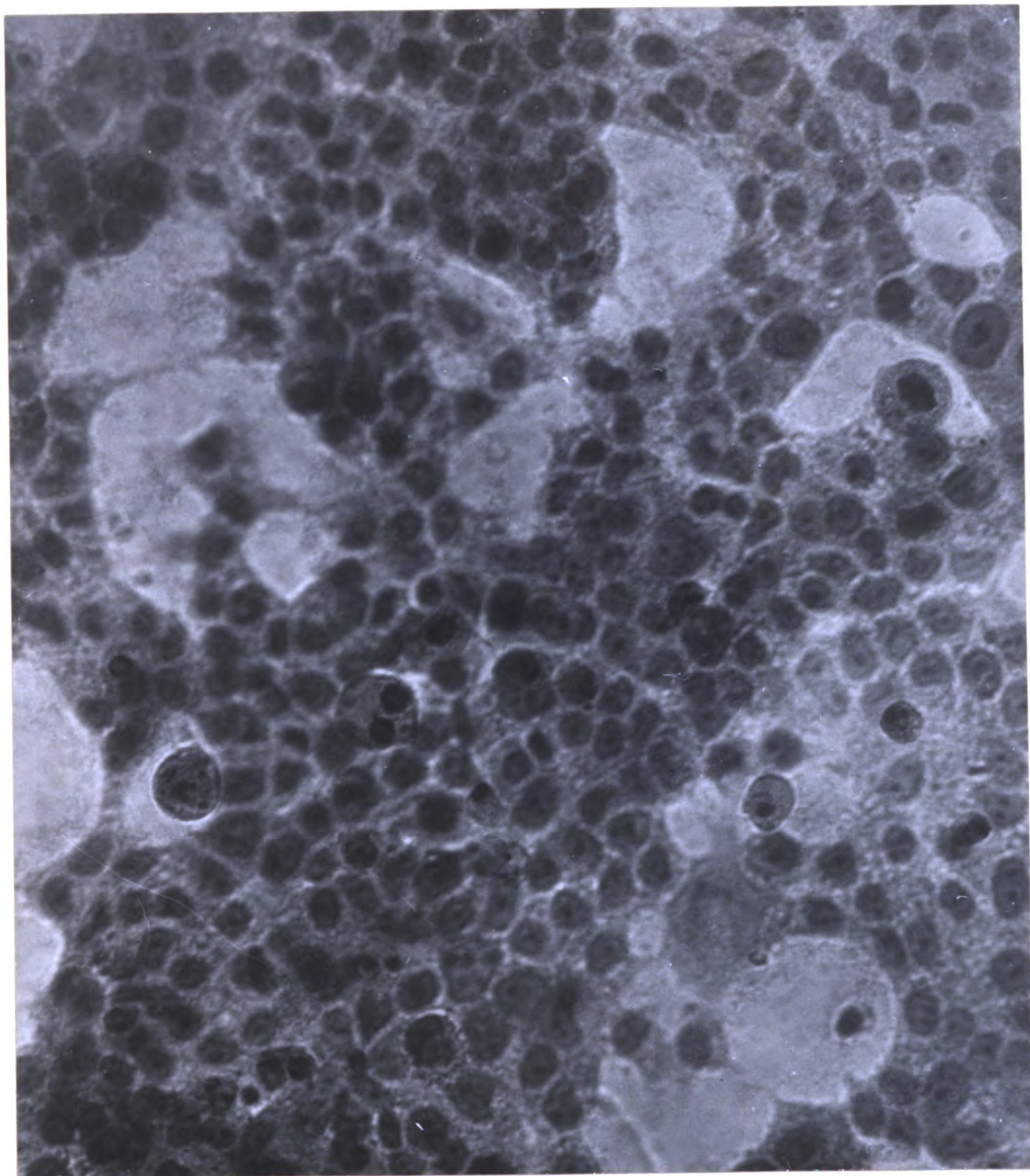
Vacuoles showing lace-like network at cell sheet margin, 10 days after inoculation. (600x)

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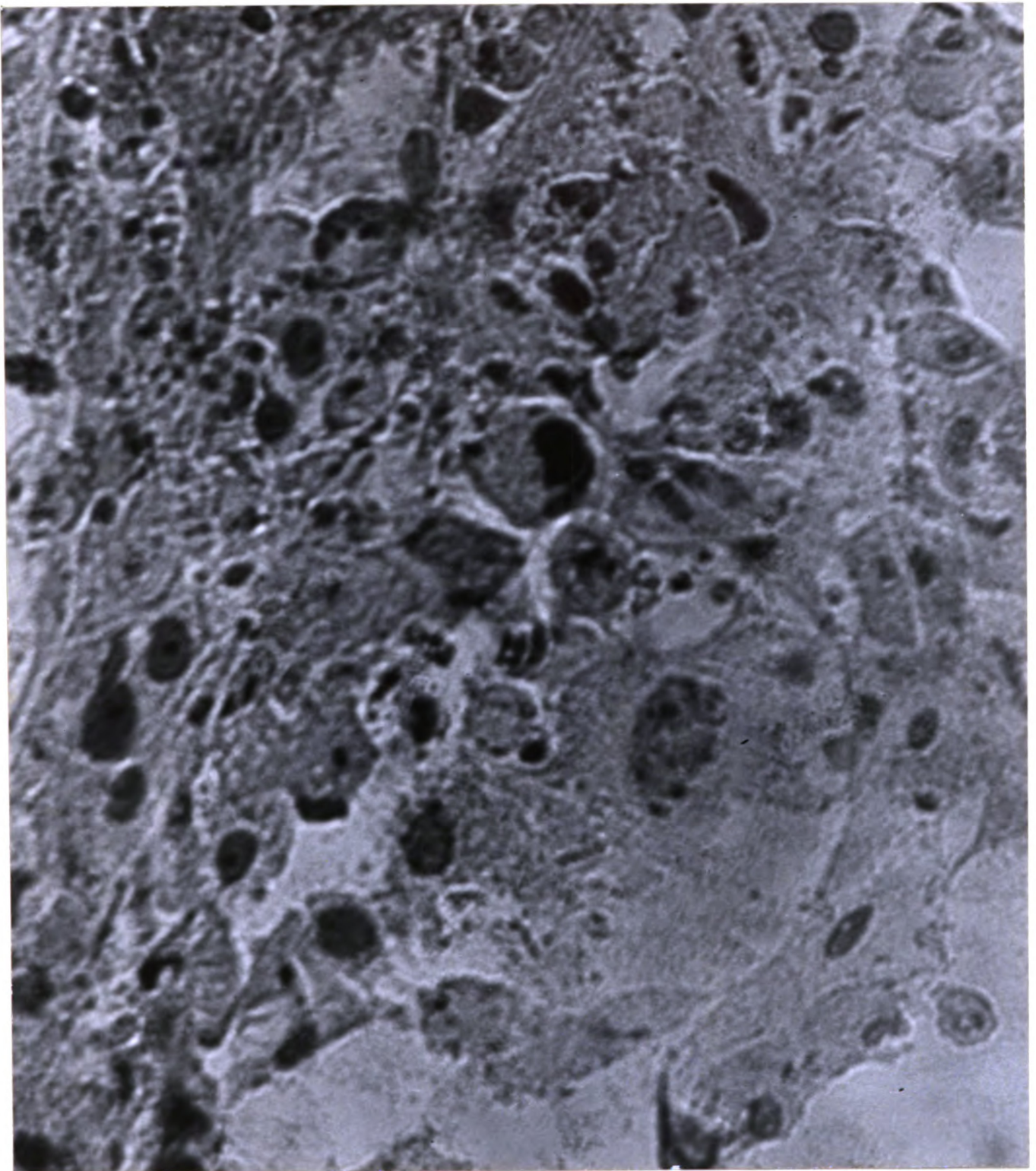
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Figure 11



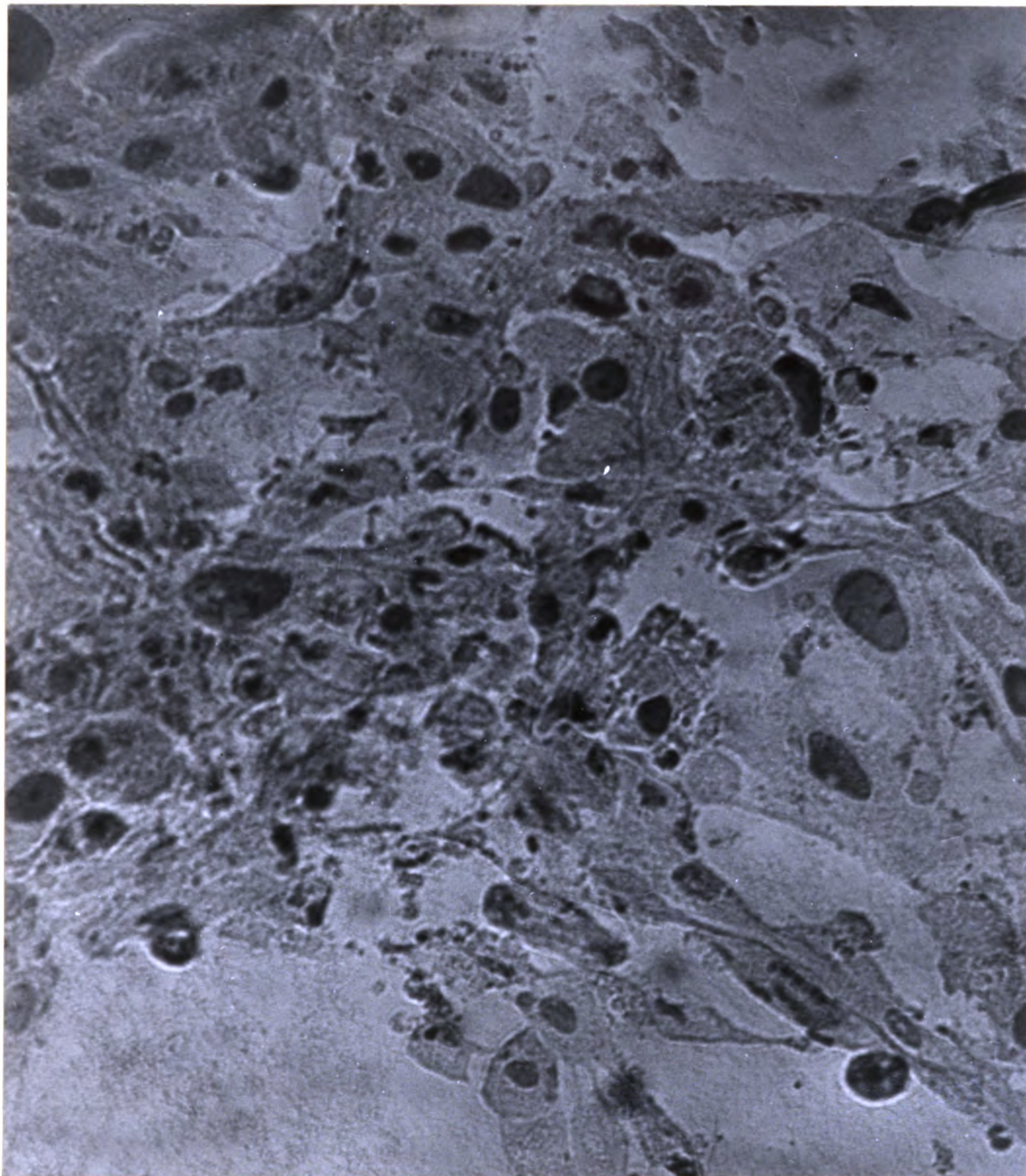
Outgrowth of normal monkey kidney cells stained with
H. & E. Control for Fig. 12, 13 and 14. (1200x)

Figure 12



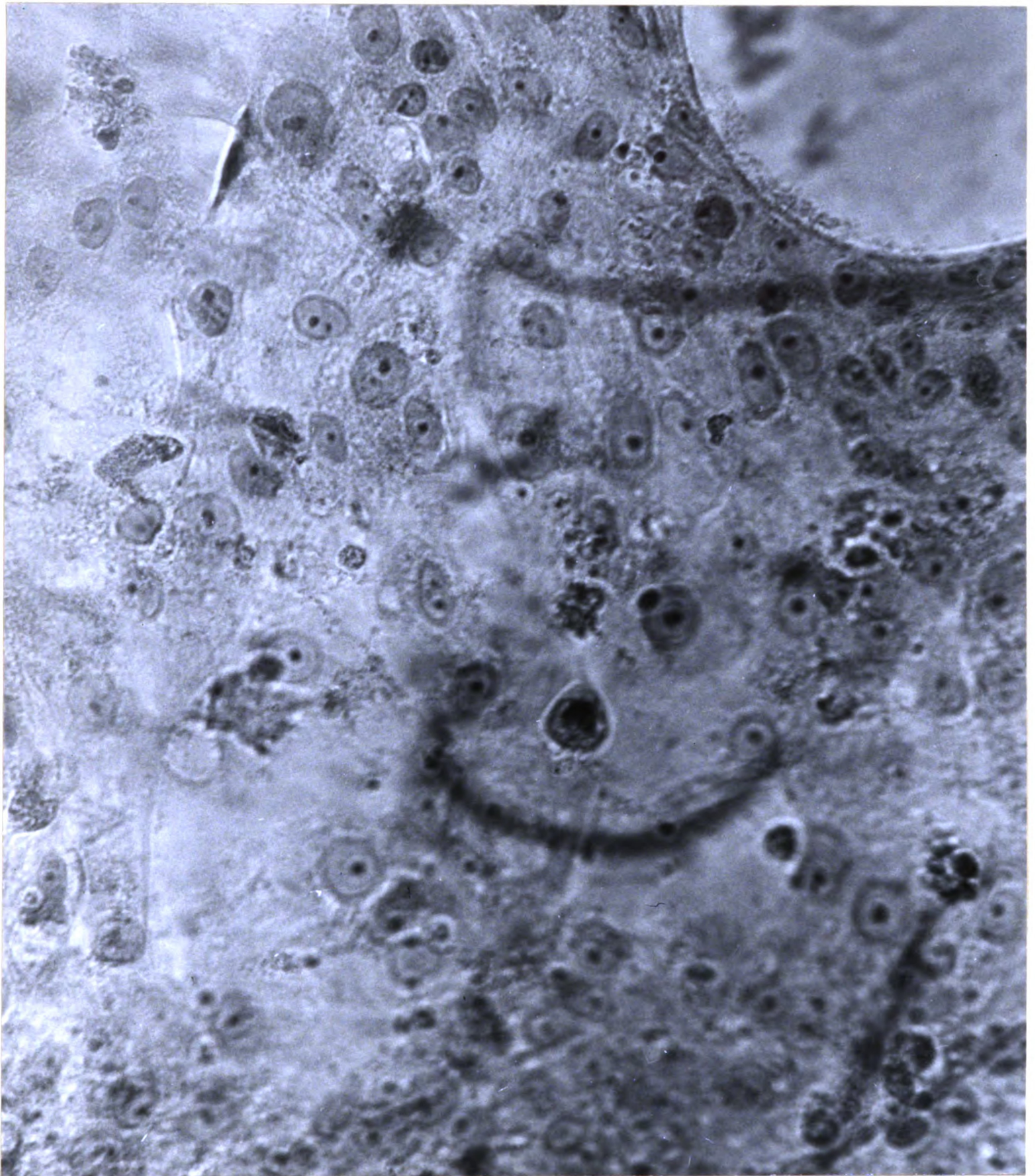
Giant cell formation and nuclear changes, 7 days
after inoculation, H. & E. stain. (1200x)

Figure 13



Giant cell formation and nuclear changes, 7 days
after inoculation, H. & E. stain. (1200x)

Figure 14



Nuclear changes of the cells around the vacuoles, 10 days after inoculation, H. & E. stain. (1200x)

DISCUSSION

Aerosols created by drinking fountains are not homogeneous but consist of viral nuclei surrounded by varying size water particles. Each water particle may consist of a single virus particle as a nucleus, or more virus particles as many nuclei in aggregation. Humidity of the atmosphere is one of many factors which plays an important part in the physical and chemical properties of such aerosols.

As others (16) have shown and as illustrated again in this work, the decay of virus aerosol has a direct bearing upon the amount of moisture present in the air. It was for this reason that the atmosphere was adjusted to represent between 50 to 70 per cent relative humidity during the collecting procedures. Under these conditions, the drinking fountain aerosol would, depending upon the relative humidity, be a marked health hazard at one time where at other times, less hazard would exist. Thus, one might expect that during summer months when the relative humidity is high, the drinking fountain would disseminate more viable virus particles than during periods of dry atmospheric conditions. Again theorizing, viruses present in the mouths of people during periods of high atmospheric moisture are the enteric viruses such as Polio, Coxsackie and the ECHO (Enteric Cytopathogenic Human Orphan) viruses and it might be possible that such viruses could be transmitted from person to person by the drinking fountain. On the other

hand, the upper respiratory infection which occurs during the dry winter months would not likely be transmitted in this manner.

The all glass impinger is not efficient in collecting virus particles below 1 μ in diameter. The amount of slippage permitted by this impinger is enormous (17). Impingement alone is known to destroy some of the virus particles. The number of virus particles recovered as shown in Tables III and IV probably represents only a small fraction of the number of particles involved in the aerosol. Both types of impingers were designed to detect experimental bacterial aerosols; they were used in this work only because of the lack of a better type of impingement. Even the air currents surrounding the drinking fountain would influence markedly the amount of virus aerosol collected.

As water splash was involved (a single drop may equal as much or more than 0.5 ml of water) and if a large drop of water was impinged, assay of the impinger broth would show high concentration of virus. This probably explains (Table III and IV) why, during aerosol collection, large amounts of virus were found in one impinger while little or no virus was demonstrated in the preceding or following impinger. These erratic results are not due to the inefficiency of different impingers of the same make. In experimental conditions in this laboratory, constant viral concentration were recovered from constant aerosol clouds.

It is certain that once the bowl of a drinking fountain becomes contaminated with a virus, continued use of the fountain increases the contamination. The above is correct if the bowl is contaminated by the act of drinking. The next step in this investigation was to determine whether use of a drinking fountain by the public contaminates the fountain to the extent whereby infectious agents could be recovered.

It can not be stated with certainty whether the cytopathogenic effect as seen in the tissue cultures was due to an infectious agent or not. The agent certainly did not produce the usual CPE as is seen with the enteric viruses. Instead, the reactions seen in the tissue cultures were localized to a comparative small area of the cell sheet. The agent did not spread to surrounding cells until the entire cell sheet was affected as is the usual case with known viruses. In many respects the CPE seen in HeLa cell cultures resembled the description given by Enders (15) with the primary isolation of the measles virus. The agents could not be recovered by serial transfer in tissue cultures thus preventing identification. The first impression was that this was measles virus and that the HeLa cell line was not suitable for primary isolation of this virus. When the questionable water samples were inoculated into monkey kidney cell cultures the reaction of the cells in this cell line was very similar to that seen in the HeLa cells.

The water supplying the drinking fountains under study,

was chlorinated. Whether the residual chlorine present in the water was sufficient to alter the viability of the agents can not be determined. Collection of the pads from each drinking fountain bowl was done after the pad had had contact with the water for 24 hours. This period might well have been too long, especially at room temperature, to permit survival of the agent or agents in question. Had the CPE been found only in monkey kidney tissue culture, one would have suspected a latent virus known to be present in such cultures (18). The changes described in this study were not those of the Monkey-Intra-Nuclear-Inclusion-Agent (MINIA) as described by Ruckle (19) as the characteristic intranuclear inclusion were not observed in stained cell preparations from either HeLa or monkey kidney cultures.

Collection of the water samples began in the middle of September. Had it been possible to make collection beginning in late June or early July when the enteric viruses are known to be at their greatest concentration in the population (20, 21 and 22), some of the enteric viruses might have been encountered. It appears that the CPE produced with the water samples in the tissue cultures used in this work was some viral agent but, because of the adverse conditions, had become denatured and was unable to propagate. The cell cultures used may not have been ideal for the virus propagation or survival and the agent's toxic effect produced a CPE similar to that described by Ackermann et al. with a strain of Type I poliovirus (23).

SUMMARY

1. By using the T-3 bacteriophage and a standard type drinking fountain, it was demonstrated that an aerosol was generated by the water splash.

2. Besides creating an aerosol, drops of water were found to splash from 12 to 21 inches out from the bowl and heavily contaminating the area around the fountain.

3. The all glass impinger was found most suitable for assay of this aerosol, the slit impinger proved to be less satisfactory.

4. A search was made for viral agents present in drinking fountains used by the public. Of 86 samples tested, 21 (24.4%) contained an agent capable of producing a localized cytopathic effect in HeLa tissue cultures. The agent producing the CPE was not recovered by additional tissue culture transfers.

5. Of the 21 water samples showing CPE on the original HeLa cell cultures only 3 produced these changes in monkey kidney tissue culture.

6. Although no virus was isolated from the water samples, there is some reasons to believe that under the proper experimental conditions and the use of different tissue culture lines, the agents encountered might be isolated and established as a virus.

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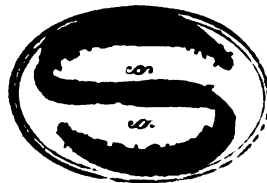
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