REACTION OF PK CELLS DURING INFECTION WITH TESCHEN DISEASE VIRUS (KLOBOUK VIRUS)

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Looking through the literature of the past decade it is surprising to find that a comparatively small number of reports appeared which dealt with histochemical studies of infected cells.

Most studies were concerned with reactions of normal cells cultivated in vitro, with reactions of cells in carcinogenetic studies, and with investigations of species differences in tissues and cultivated cells; in various normal or pathological cells and tissues enzyme activities, detection of proteins, nucleoproteins, mucopolysaccharides were investigated to a limited extent. Most recently the action of metabolic inhibitors was investigated cytochemically (Caspersson et al., 1965), and the enzymatic activity of KB cells treated with staphylococcal hemolysins (Jeljaszewicz et al.,1965) was studied by histochemical methods.

Although the usefulness of cytochemical methods for the study of infected cells was suggested by Lépine and Sautter in 1946 and by several other workers since then, subsequently only a few reports appeared that dealt with the problem. As to the techniques employed biochemical methods were chiefly used, save for the histochemical studies of some enzymes in normal HeLa cells by Fortelius, Levonen and Saxén (1961). The reaction of infected cells was studied cytochemically by Pollard et al.,(1960) in the model of psittacosis using acridine orange staining and fluorinated pyrimidines. Employing quantitative biochemical methods Kovács et al.(1956-60) stu-

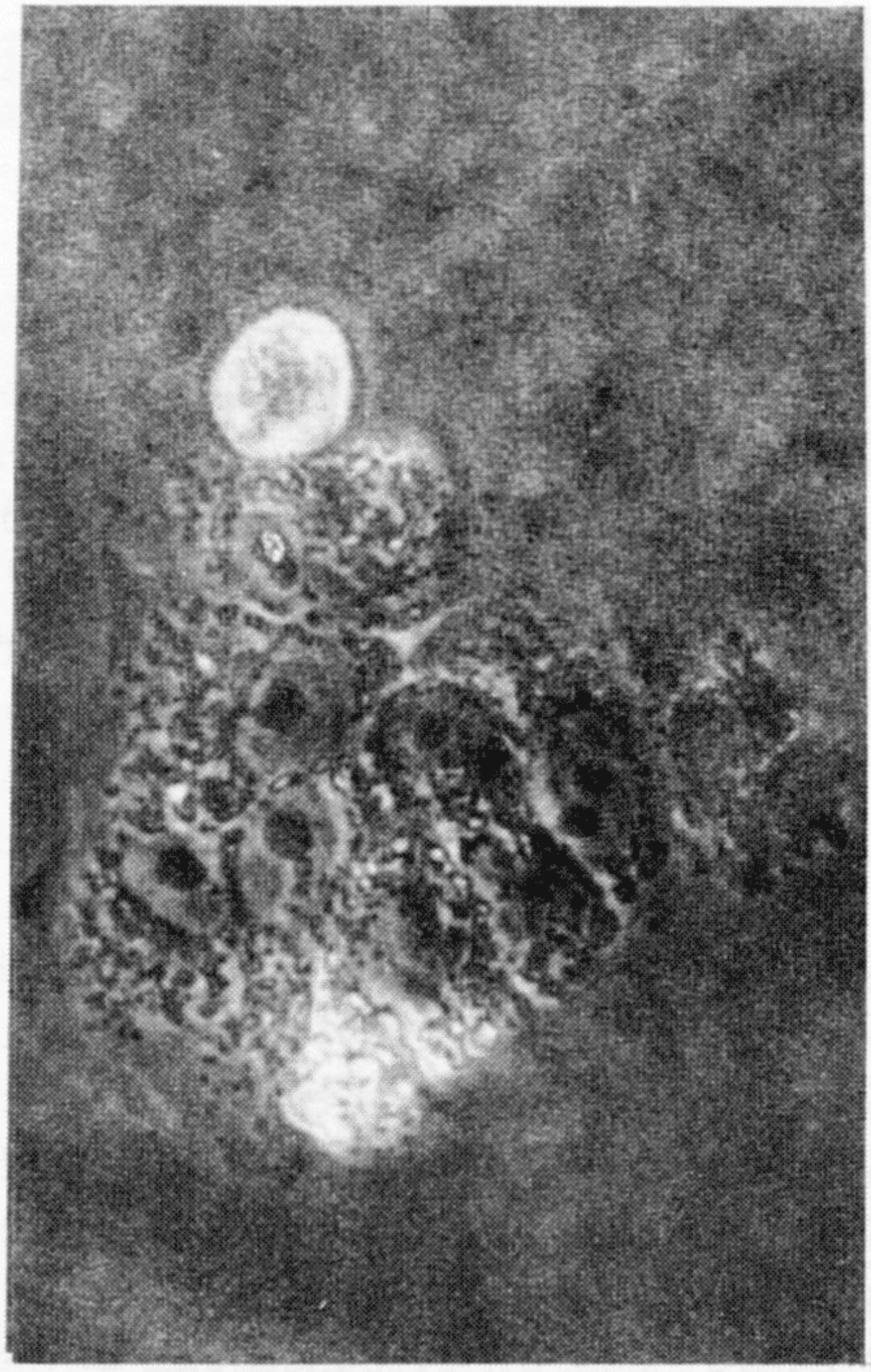

Fig. 10

died the fate of hydrolases in the HeLa cell, or rhesus monkey kidney cell - poliovirus model. Kelly, Greiff and Anderson (1962) studied histochemically mucopolysaccharides and mucoproteins in influenza virus-infected embryonate eggs in relation to changes in sialic acid levels. Felgenhauer and Stammler (1962) made histochemical studies of the dehydrogenase activities, of proteins, lipids, mucopolysaccharides and nucleic acids in herpesvirus-infected L cells. Morris (1962) studied histochemical changes in the virus-infected western oak looper. Godman et al. (1964) made a very detailed study of the biochemical events in ECHO-9 virus-infected rhesus monkey kidney cells, using histochemical and biochemical methods.

In this round-up of the literature we are intentionally omitting the several earlier reports in which only one or two reactions were employed, such as the Feulgen reaction and hematoxylin-eosin staining, or where only the fate of nucleic acids in infected cells was investigated (e.g.acridine orange staining).

We have attempted to employ histochemical procedures to determine the qualitative changes which occur in stable line pig kidney epithelial cells (PK) as a result of infection with Teschen disease virus (TDV) belonging to the picornavirus group (Patočka, Kubelka and Korych, 1959).

In previous studies (Korych, Chýle and Franěk, 1965) the kinetics of multiplication of TDV, the production of extracellular virus, the onset and development of cytopathic changes, and the presence of intracellular viral antigen by the fluorescent antibody technique have been described.These results are summarized in Figure 1. Free virus appears in significant amounts after 8 hours following infection of cells. The progress of cytopathic changes is in line with the appearance of extracellular virus. Within the first 14 hours the curve representing the appearance of intracellular fluorescent granulations is biphasic in shape, its first peak appearing at about 5 to 8 hours, and the second peak at about 10 to 12 hours.

This corresponds to four-hour cycles of intracellular development of virus. Figures 2 to 6 illustrate the development of cytopathic changes in PK cell tube cultures at 0, 5, 9, and 18 hours after infection as compared with a control. In the phase contrast the first really conspicuous signs of cellular alteration were apparent at about 7 hours after infection.

On the basis of these findings it has been attempted to determine what changes occur in the enzymatic activity of thes infected cells and to see whether and what parallel can be found under similar experimental conditions, and whether a reaction can be found that might perhaps be specific for the viral infection of the cell.

Two designs were adopted for this study. Firstly,young and intentionally non-confluent cultures of PK cells were used Cells were prepared by seeding trypsinized suspensions into larger Petri dishes containing glass squares cut from microscope slides. The dishes were sealed in larger vessels without any further attempt to regulate the composition of the atmosphere. The cells were cultivated for 1 to 3 days at the most. These glass-slide cell cultures were then infected at various intervals: 5, 9, 18 hours, terminated, and histochemically processed simultaneously. Secondly, confluent cell cultures on glass slides, prepared in an identical manner, were infected with virus, and overlaid with agar maintenance medium. The overlaid cells were incubated for only 72 hours, that is for a shorter period than that used in the plaque assay technique developed for this virus (Korych and Chýle, 1965) in order to avoid, or at least limit the development of nonspecific changes occurring after prolonged maintenance of cells under agar overlay.

The virus used in these studies was a nonvirulent strain A3b of the Teschen disease virus in its 105^{th} to 120^{th} PK cell passage, developed and cloned in this laboratory.

Cultures assayed at 5, 9 and 18 hours after infection were inoculated with o.03 ml of concentrated virus suspension per slide ($10^{8.5}$ $TCID_{50}$ per ml). Agar overlaid slide cultures

were inoculated similarly with the same virus suspension diluted 10^{-3}.

The PK cells were received in their 32nd passage in 1958 and maintained ever since. The cells were in their 176th passage when first used in histochemical experiments four years ago; in recent experiments the cells were already past their 370th passage.

Culture media consisted of modified Earle's saline supplemented with 0.5% lactalbumin hydrolysate and 10% bovine serum.

Infected and control slide cultures were stained with Giemsa, toluidine blue, azure A, and methyl green-pyronin. The Feulgen reaction, sudan black B staining, the periodic acid-Schiff and the alcian blue reaction were performed. Infected and control cells were also assayed histochemically for alkaline and acid phosphatases, for adenosine-triphosphatase, adenosine-monophosphatase and thiamine pyrophosphatase; for nonspecific esterases; for succinate dehydrogenase, NAD tetrazolium reductase(DPN diaphorase), lactate and beta-hydroxybutyrate dehydrogenases; and for isocitrate and glucose-6-phosphate dehydrogenases.(Lojda and Papoušek, 1966).

The results obtained by these methods can be outlined by arranging them into four groups.

Group I in which infected cells as compared with controls (both being positive) did not show any significant differences when tested for (a) the Brachet reaction (methyl green-pyronin), in both cases cells were positive, more intensive in smaller cells after 18 hours, after 72 hours infected focuses stained more; (b) toluidine blue gave similar results; (c) nonspecific esterase after 5, 9, and 18 hours; (d) Fettrot: the cytoplasm was negative, only remains from growth medium on cell surfaces were found, in infected slides fat droplets were present in pyknotic cells; (e) acid phosphatase with naphtyl ASBO phosphate; (f) ATPase and AMPase.

Group II. Only slightly more marked activities were apparent in infected cells than in controls when tested for (a) the

periodic acid-Schiff reaction, generally, however, the reaction was more intensive in smaller cells; (b) alkaline phosphatase; (c) DPN diaphorase; (d) AS esterase; (e) after staining with sudan black B smaller cells were more intensively colored; (f) thiamine pyrophosphatase after 5 hours.

Group III. In reactions for (a) nonspecific esterase after 72 hours control cultures (Fig.7) evinced higher activities, in infected cultures (Fig.8) only focuses of cytopathic changes were more intensively colored, while the rest of the cells were less reactive than in the controls ! (b) The Feulgen reaction gave a "calmer" picture than controls, in infected slides the cytoplasm was more reactive.

Group IV. The greatest differences were found in reactions for dehydrogenases, showing that during infection the redox processes are greatly activated. According to the intensity of the reactions these can be arranged in the following order: the most intensive staining was obtained with lactate dehydrogenase (Fig. 9 to 13), then with isocitrate, succinate, glucose-6-phosphate, and beta-hydroxybutyrate dehydrogenases.

The intensity of the activity of acid phosphatases, especially with alpha-naphtyl phosphate was remarkable. In all intervals tested the activities in infected cells were increased. (Fig. 14 and 15).

As for the present, we are not able to distinguish whether the altered enzymatic activities detected in Teschen disease virus-infected cells represent a specific reaction, or whether they are part of the general response of any cell, or of certain types of cells to noxious agents and infectious particles as a whole.

The determination of the nature and significance of these responses is the subject of our further investigations, employing other cell systems and other viruses.

The usefulness of cytochemical methods in the study of virus-infected cells has been tested by several investigators. Greiff's studies suggest even more. And so one day when laboratory workers will be able to detect more than the 63 enzymes

detectable by present histochemical techniques out of the 700 or so thus far listed, we believe it will be possible to detect indirectly the presence of viruses in cells in the earlier stages of infection by these methods.

Abstract

It has been attempted to demonstrate the response of PK cells to infection with Teschen disease virus by histochemical techniques.

The enzymatic activities of nonconfluent cell cultures infected at various intervals, and infected confluent cell cultures overlaid with agar were assayed. Histochemical changes were best observable in assays for acid phosphatase and dehydrogenase activities. Agar cultures presented a picture of gradation of the reaction in focuses of cytopathic changes or in cells surrounding sites of plaque formation. Agar cultures tested parallelly serve as a convenient control and guide in the evaluation of cnanges taking place in the cell cultures tested at intervals preceding completion of cycles of viral development.

References

Caspersson, T., Farber, S., Foley, C.E., Killander, D., and Zetterberger, A.
Exp. Cell Res., 39 : 365-385, 1965.

Felgenhauer, K., and Stammler, A.
Arch. ges. Virusf., 12 : 223-232, 1962.

Fortelius, P., Levonen, E., and Saxén, E.
Acta path. microb. scand., 52 : 23-33, 1961.

Godman, G.C., Rifkind, R.A., Page, R.B., Howe, C., and Rose, H.M.
Amer. J. Pathol., 44 : 215-245, 1964.

Jeljaszewicz, J., Szmigielski, S., Korbecki, M. and Zak, C.
J. infect. Dis., 115 : 421-428, 1965.

Kelly, R., Greiff, D., and Anderson, D.
J. Immunol., 89 : 815-822, 1962.

Korych, R., Chýle, M., and Franěk, J.
J. Hyg. Epidemiol. Microbiol. Immunol. (Prague), 9 : 31-36, 1965.

Korych, B., and Chýle, M.
J. Hyg. Epidemiol. Microbiol. Immunol. (Prague), 9 : 37-42, 1965.

Kovács, E.
Proc. Soc. Exp. Biol. Med., 92 : 183-188, 1956.

Kovács, E., Wagner, G., and Stürtz, V.
Z. Naturforsch., 15b : 507-517, 1960.

Lépine, P., and Sautter, V.
Ann. Inst. Pasteur, 72 : 174-183, 1946.

Lojda, Z., and Papoušek, F.
"Skripta pro účastníky kursu z histochemie enzymů", Brno, 1966.

Morris, O.N.
J. Insect Pathol., 4 : 454-464, 1962.

Patočka, F., Kubelka, V., and Korych, B.
J. Hyg. Epidemiol. Microbiol. Immunol. (Prague), 3 : 1-15, 1959.

Pollard, M., Moore, R.W., Tanami, Y., and Starr, T.J. Texas Rep. Biol. Med., 19 : 343-351, 1961.

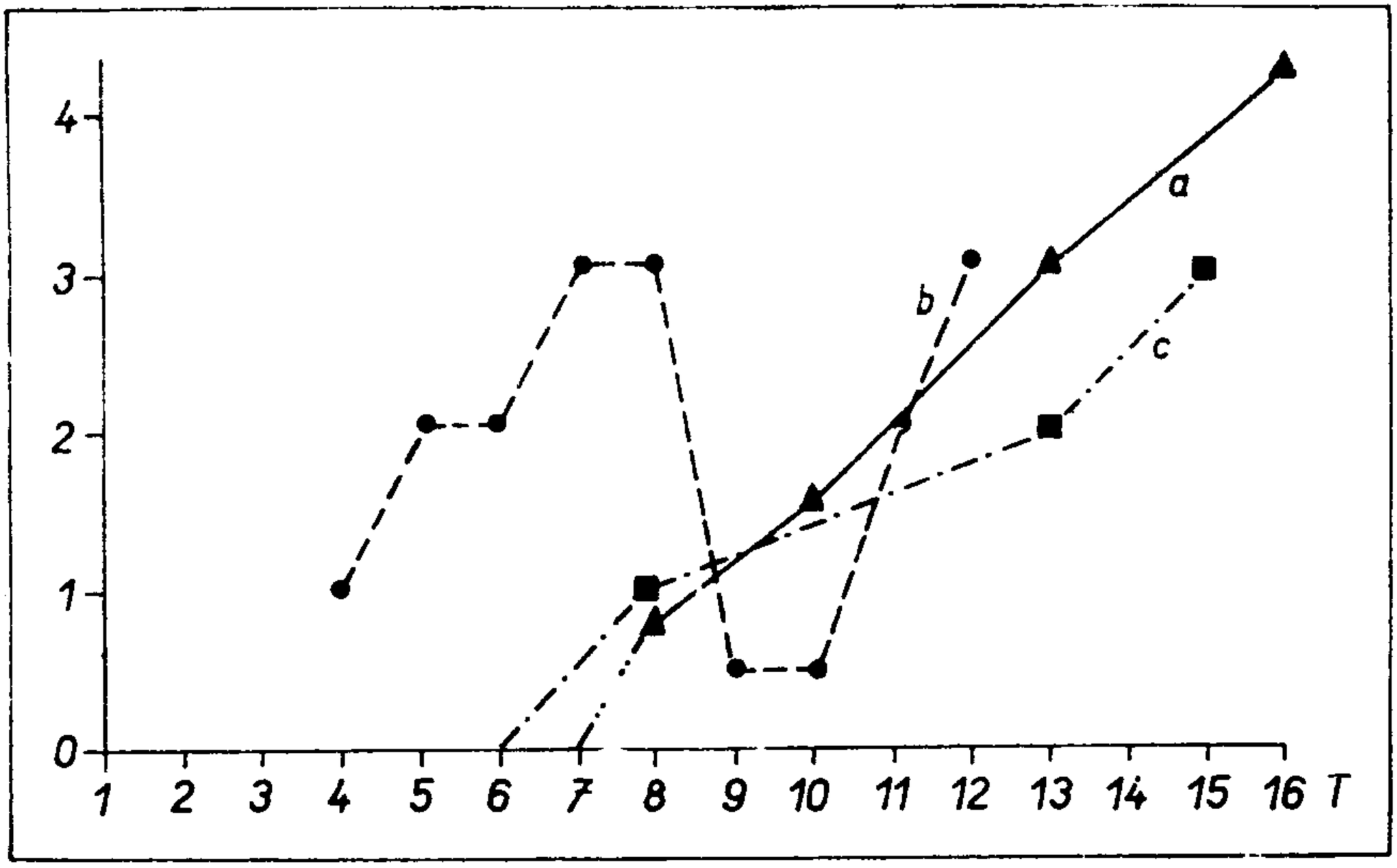

Figure 1.

Development of Teschen disease virus in PK cells.
Curves a: extracellular virus (log $TCID_{50}$);
b: intensity of intracellular fluorescence (max.+++);
c: cytopathic changes (max.++++).

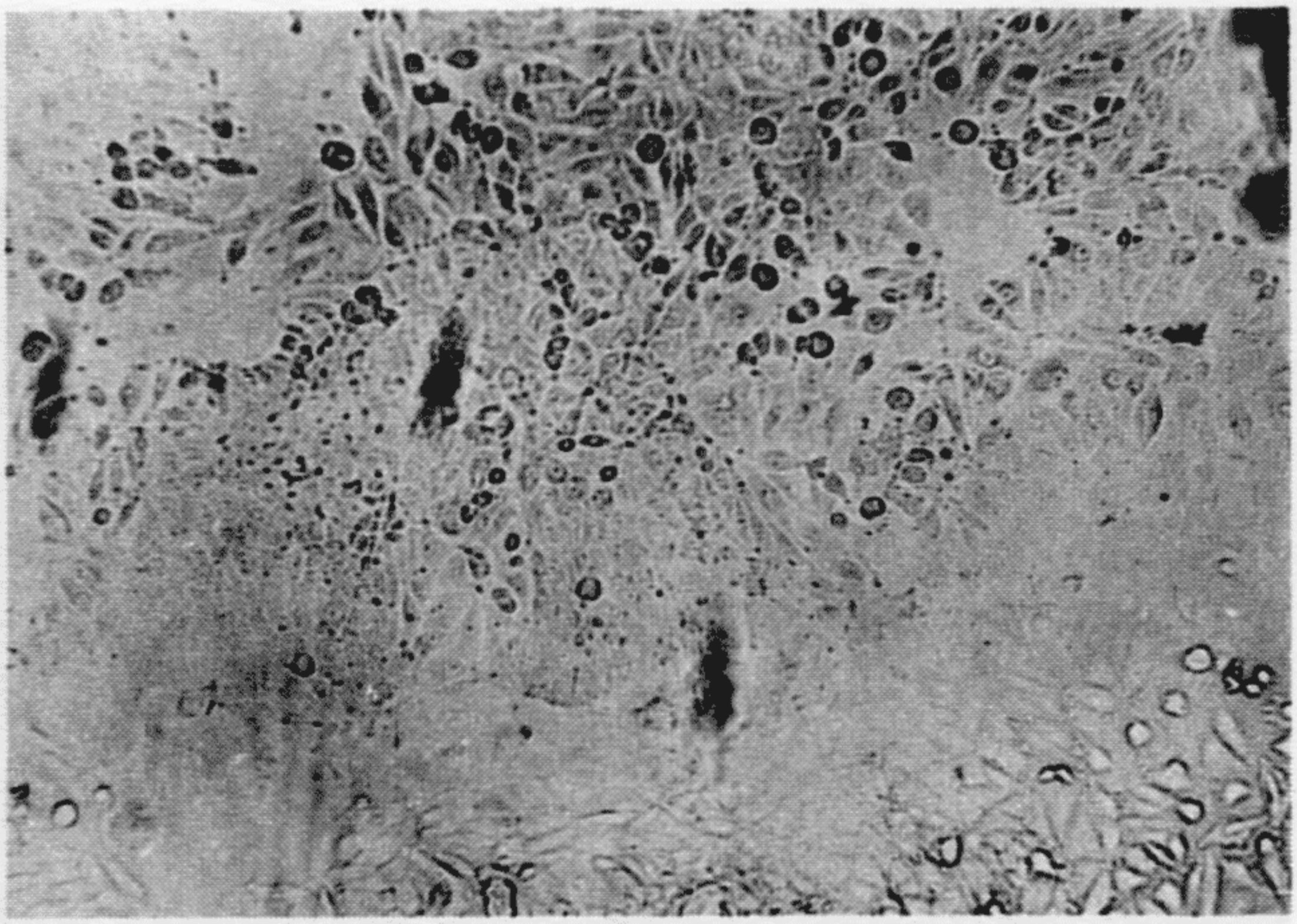

Figure 2.

Uninfected PK cells, tube culture, 18 hrs. (60x).

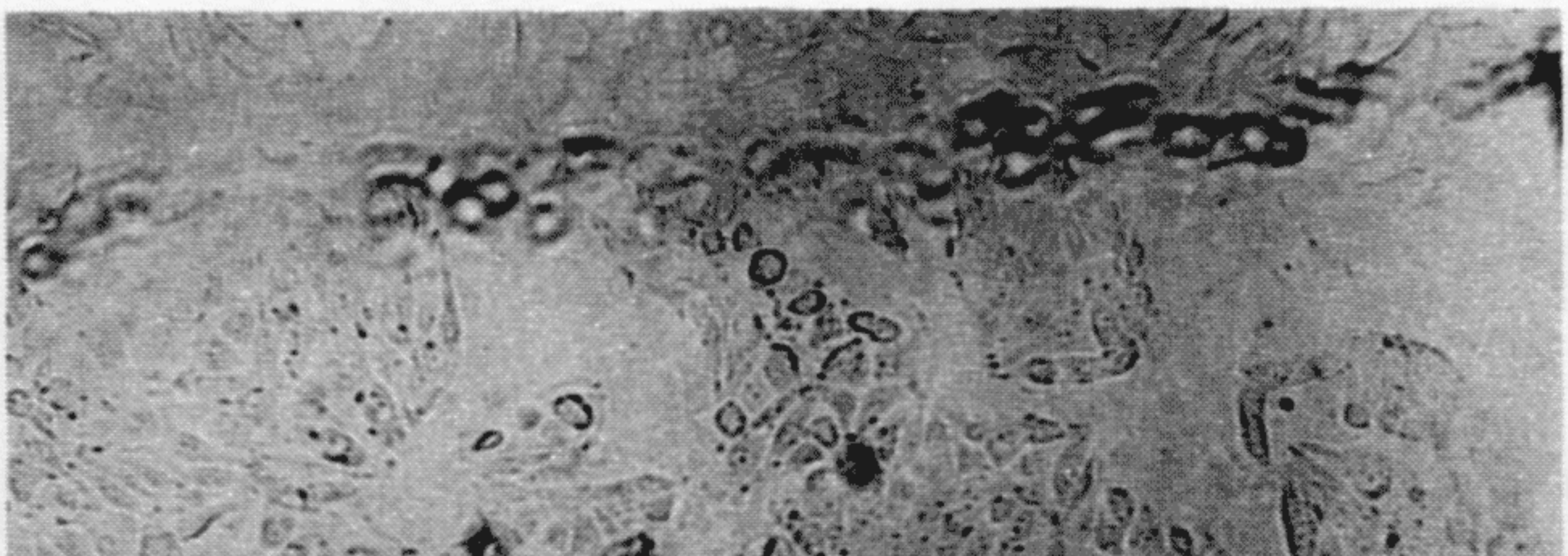

Figure 3.

TDV-infected PK cells, tube culture, 0 hr. (60x).

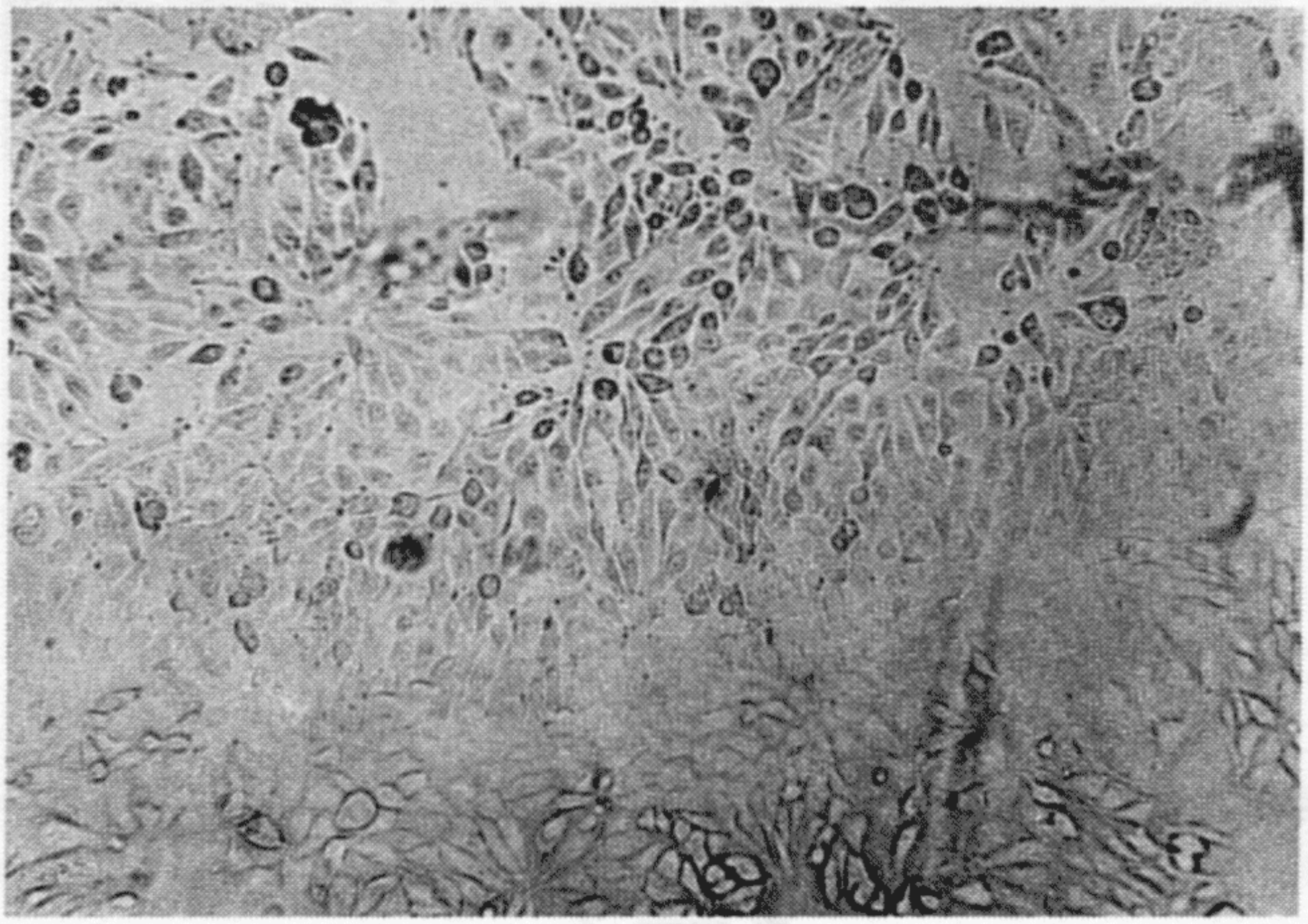

Figure 4.

TDV-infected PK cells, 5 hrs. (60x).

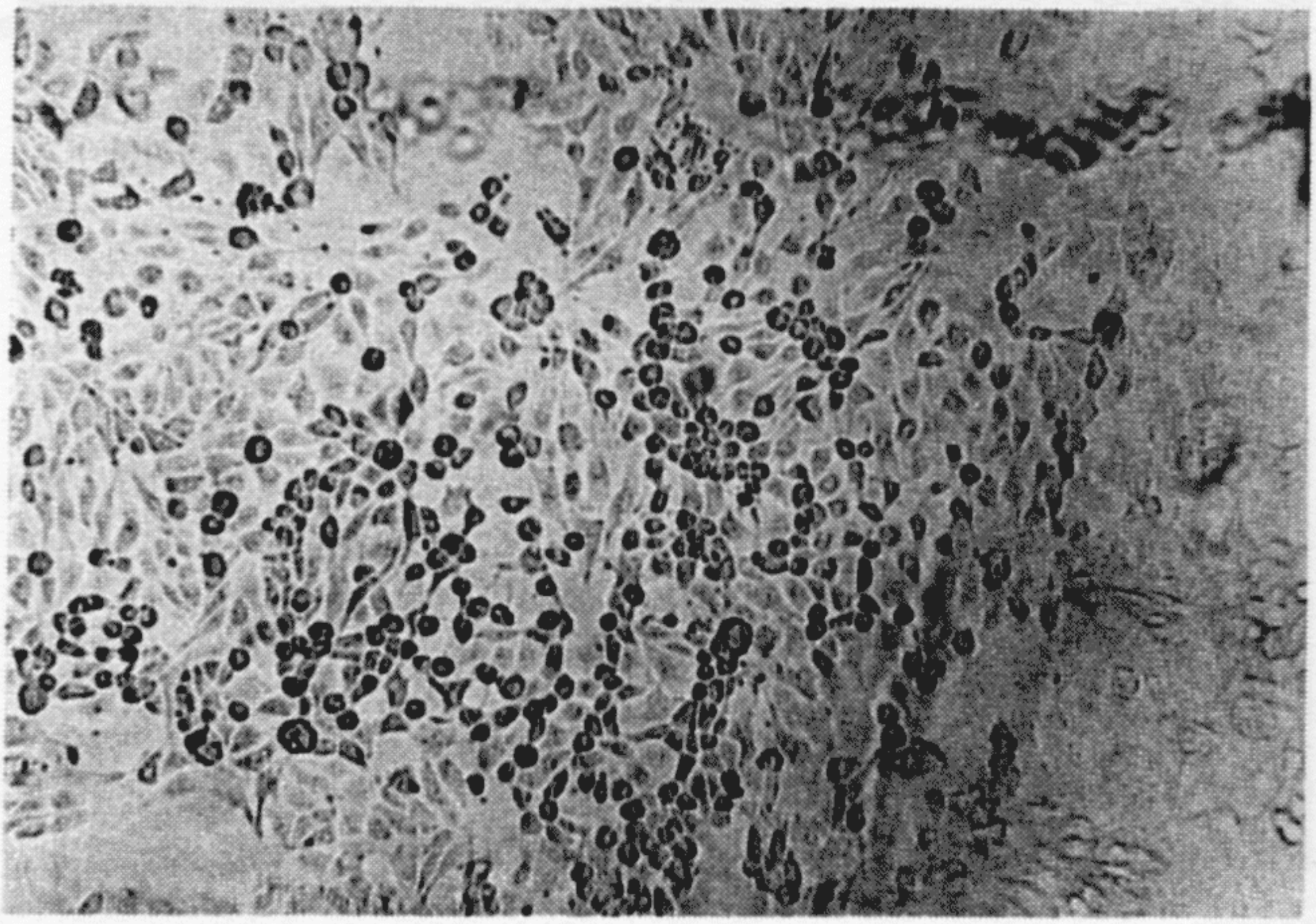

Figure 5.

TDV-infected PK cells, 9 hrs. (60x).

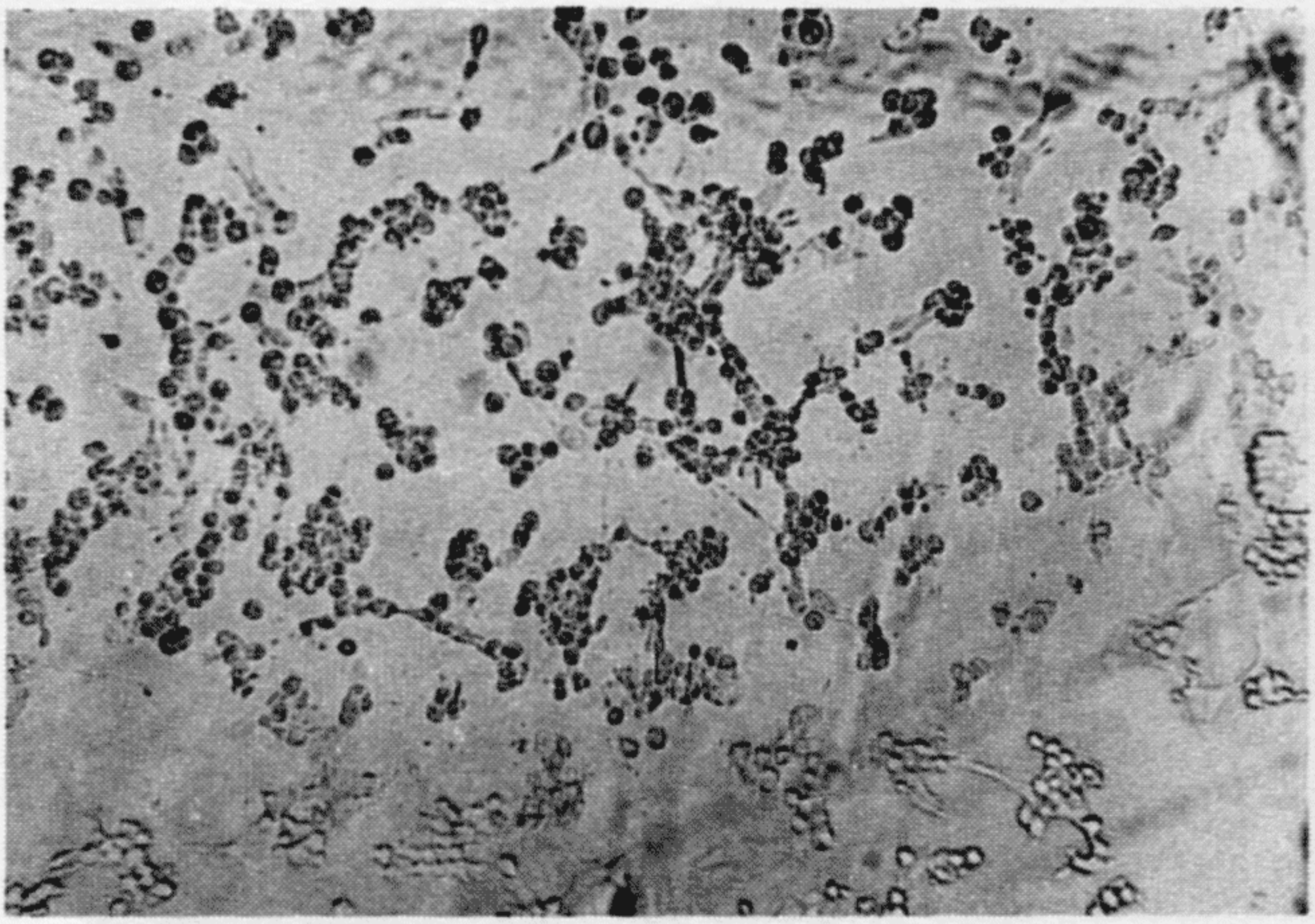

Figure 6.

TDV-infected PK cells, 18 hrs. (60x).

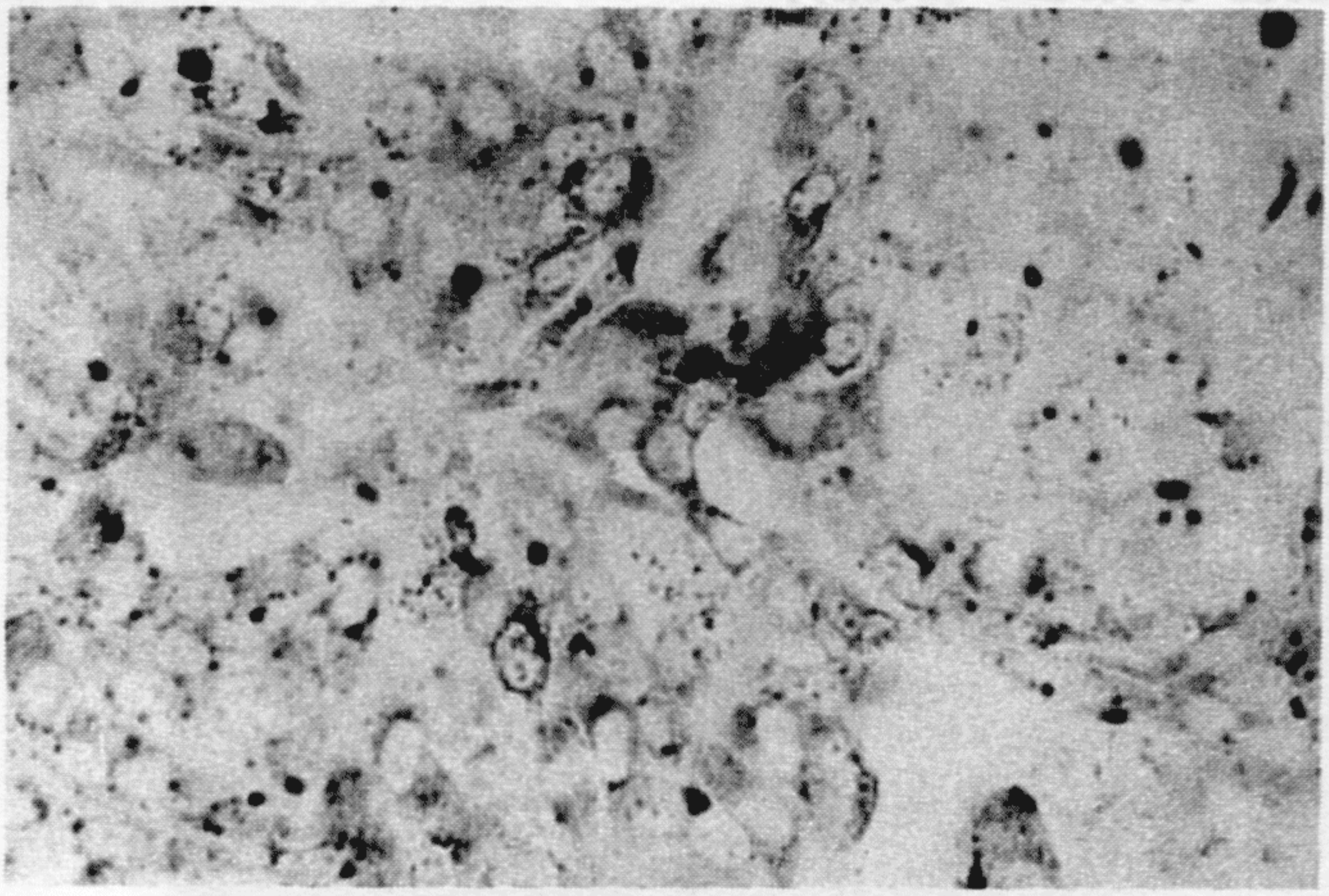

Figure 7.

Control PK cell slide culture overlaid with agar for 72 hrs, test for nonspecific esterase (alpha-naphtyl acetate, hexazo-p-rosanilin), (200x).

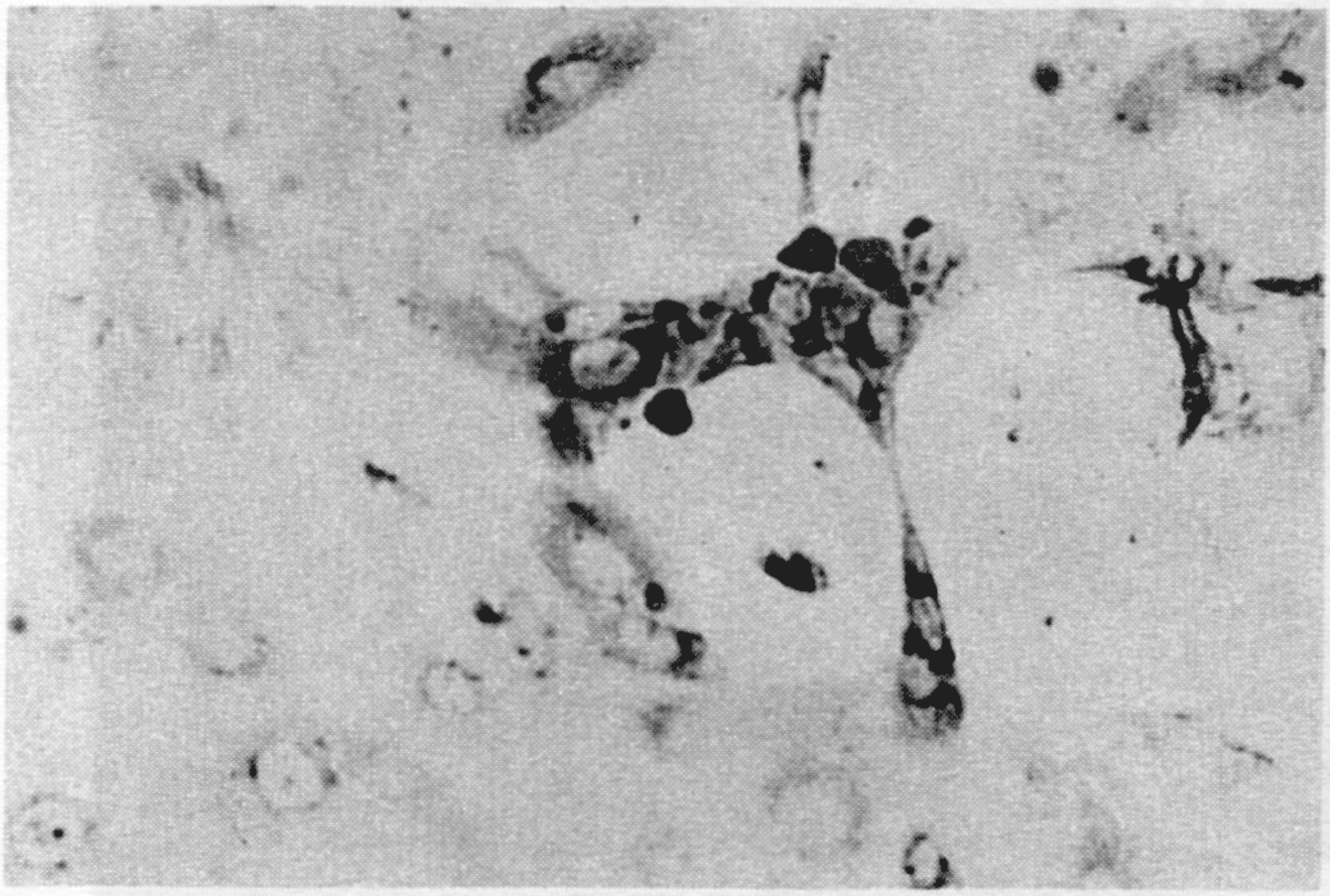

Figure 8.

TDV-infected agar-overlaid PK cells at 72 hrs, test for nonspecific esterase (200x).

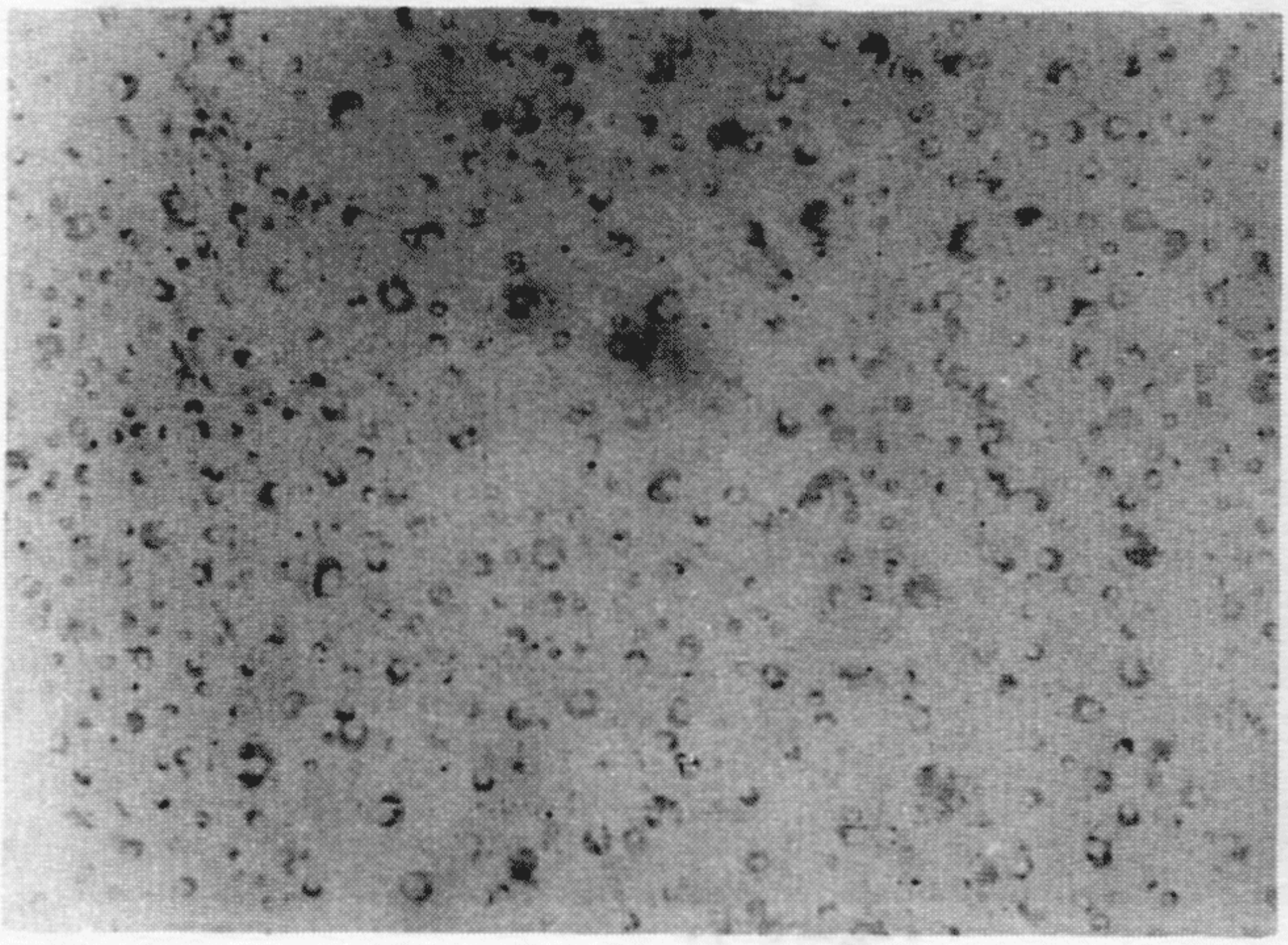

Figure 9.

Control agar-overlaid PK cells at 72 hrs, lactate dehydrogenase (nitro BT), (60x).

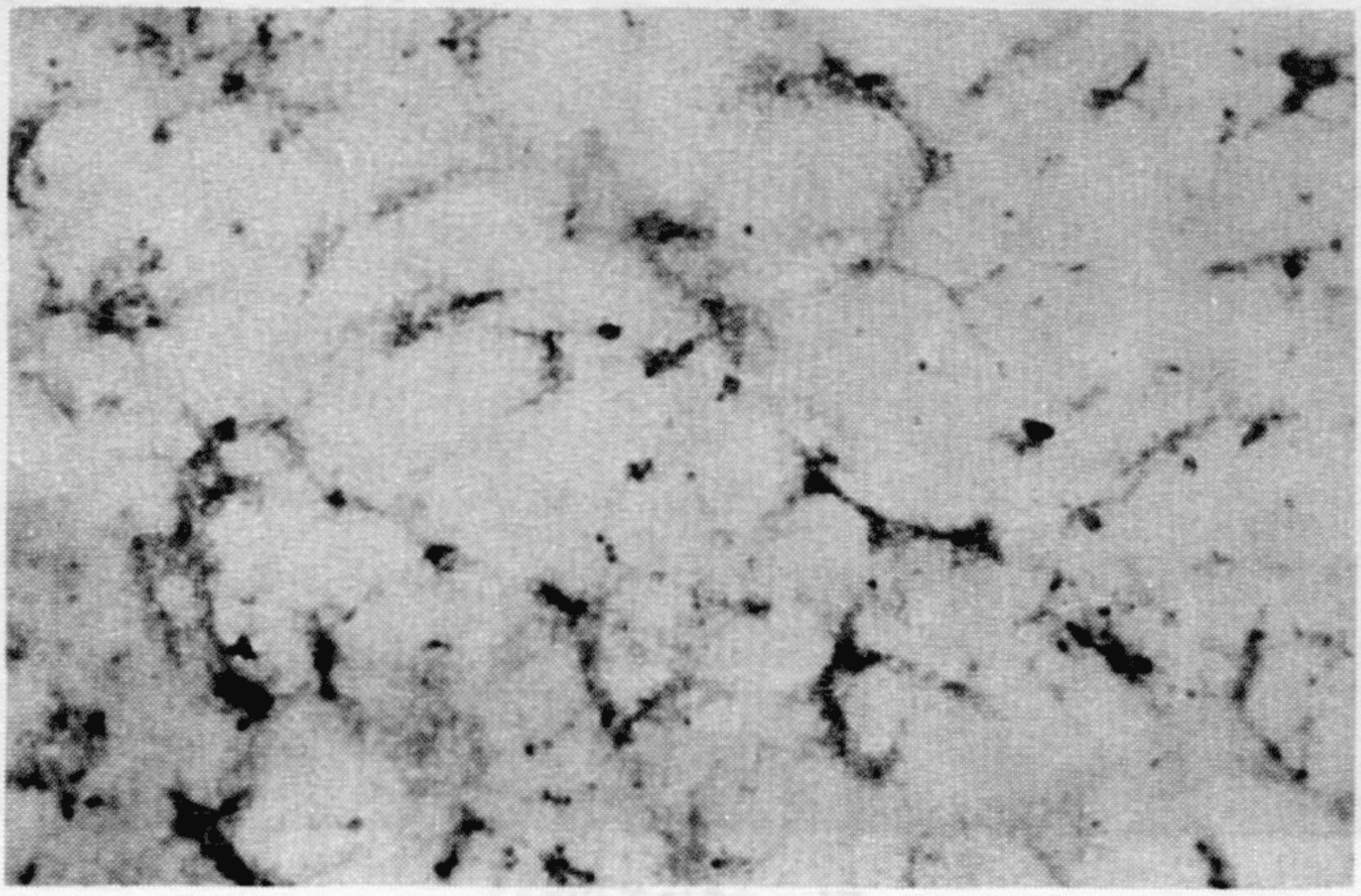

Figure 10.

TDV-infected agar-overlaid PK cells at 72 hrs, lactate dehydrogenase (60x).

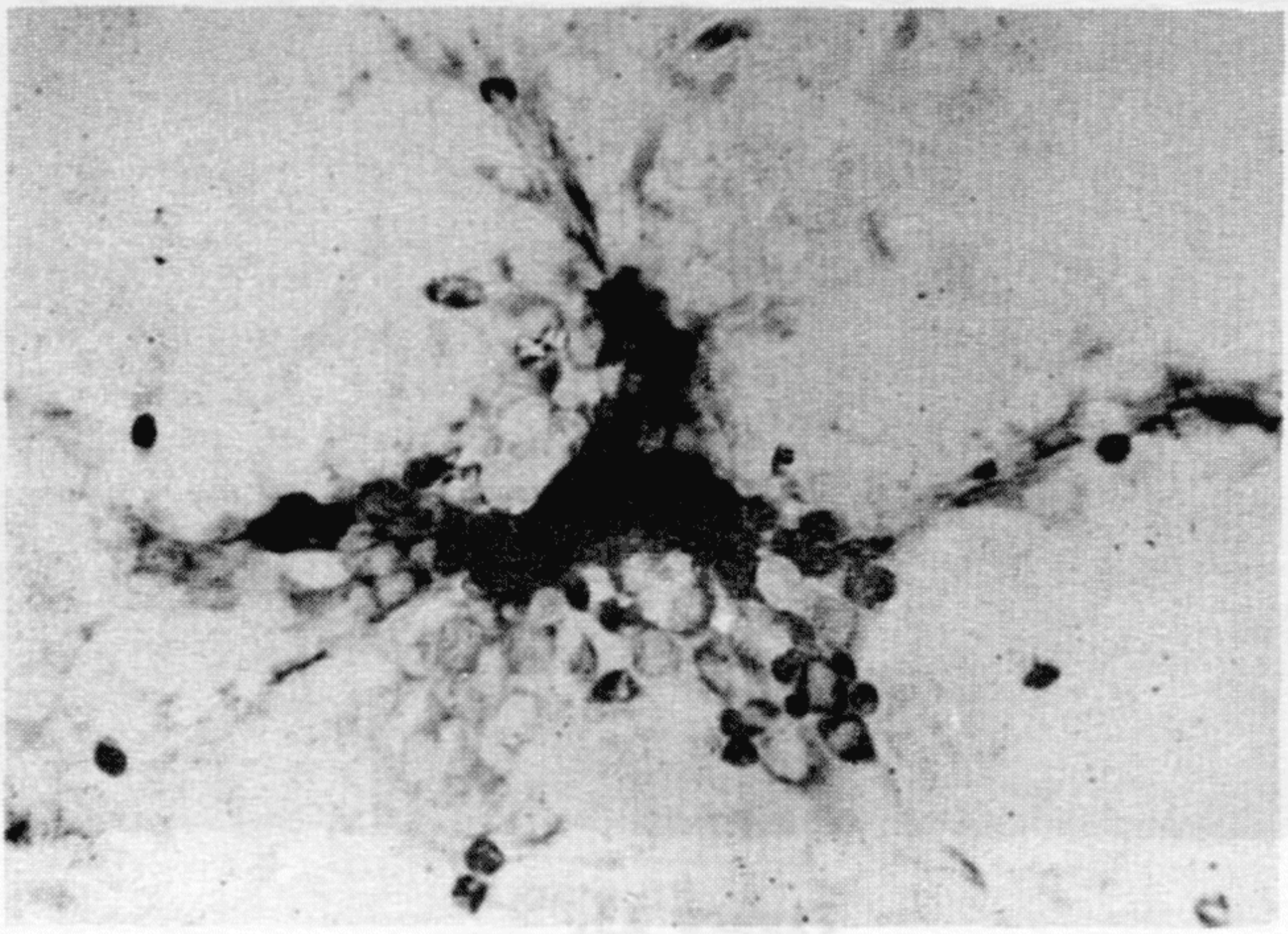

Figure 11.

TDV-infected agar-overlaid PK cells at 72 hrs, lactate dehydrogenase (200x).

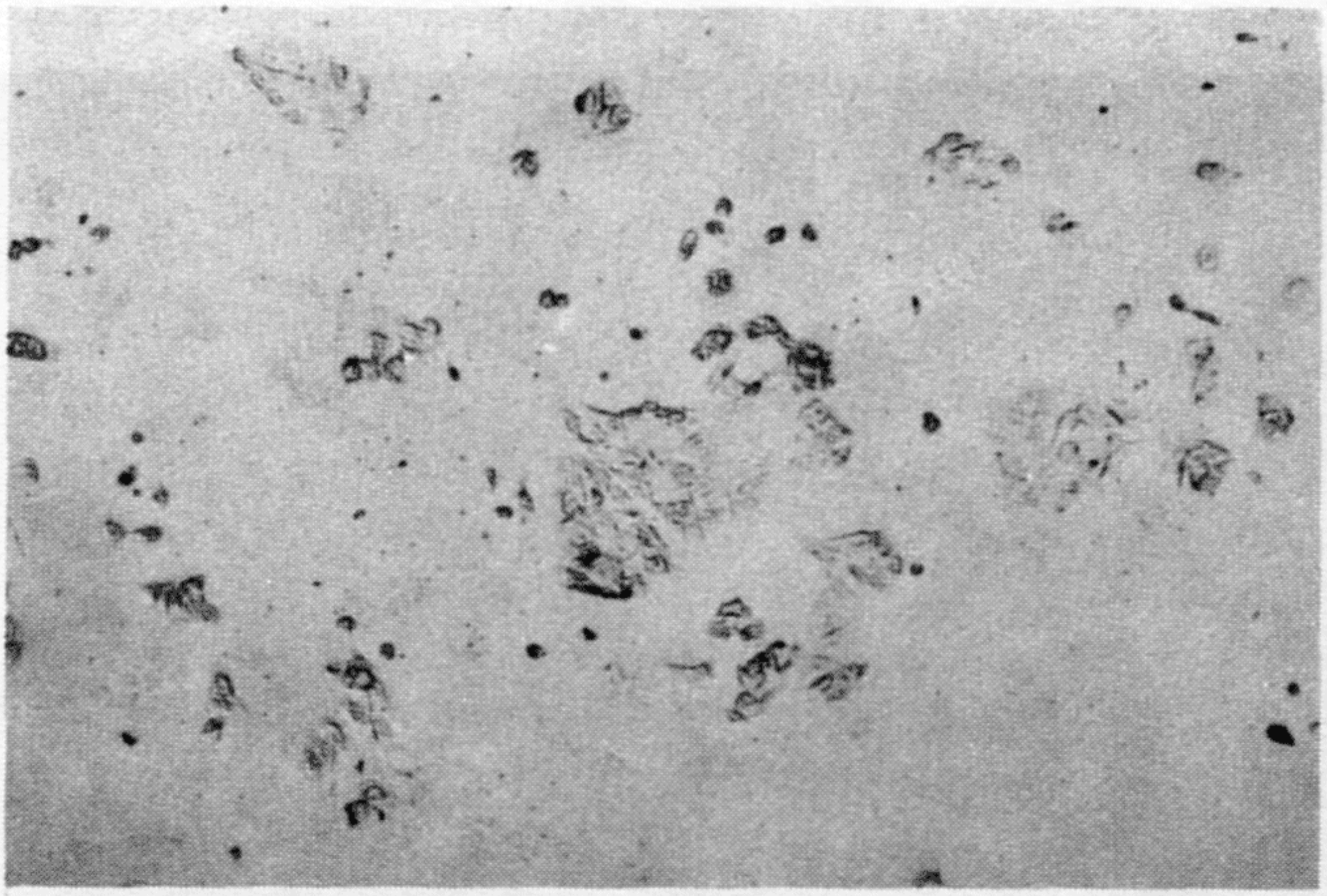

Figure 12.

Control PK cell slide culture after sham inoculation, lactate dehydrogenase (60x).

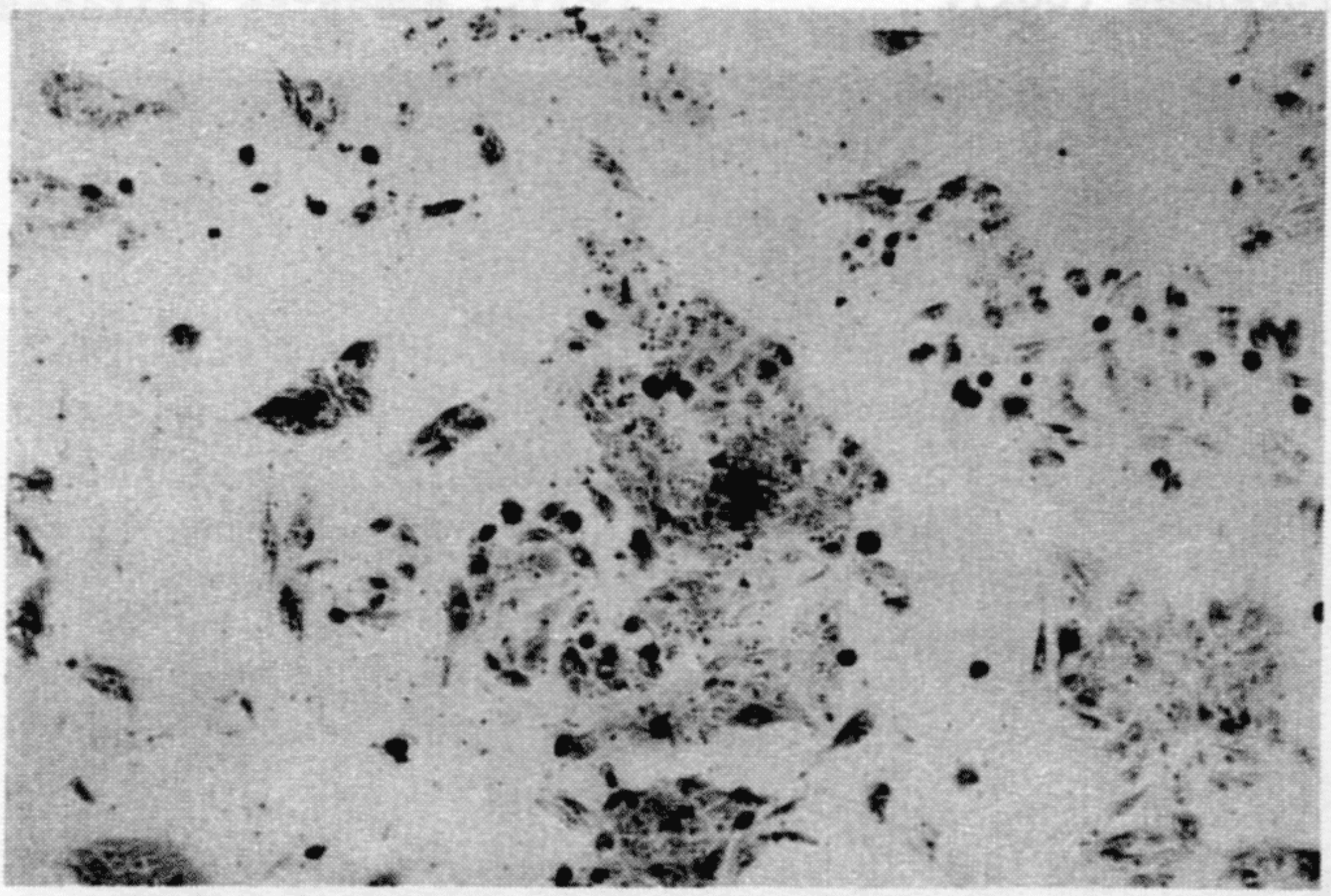

Figure 13.

TDV-infected cells at 9 hrs post inoculation, lactate dehydrogenase (60x).

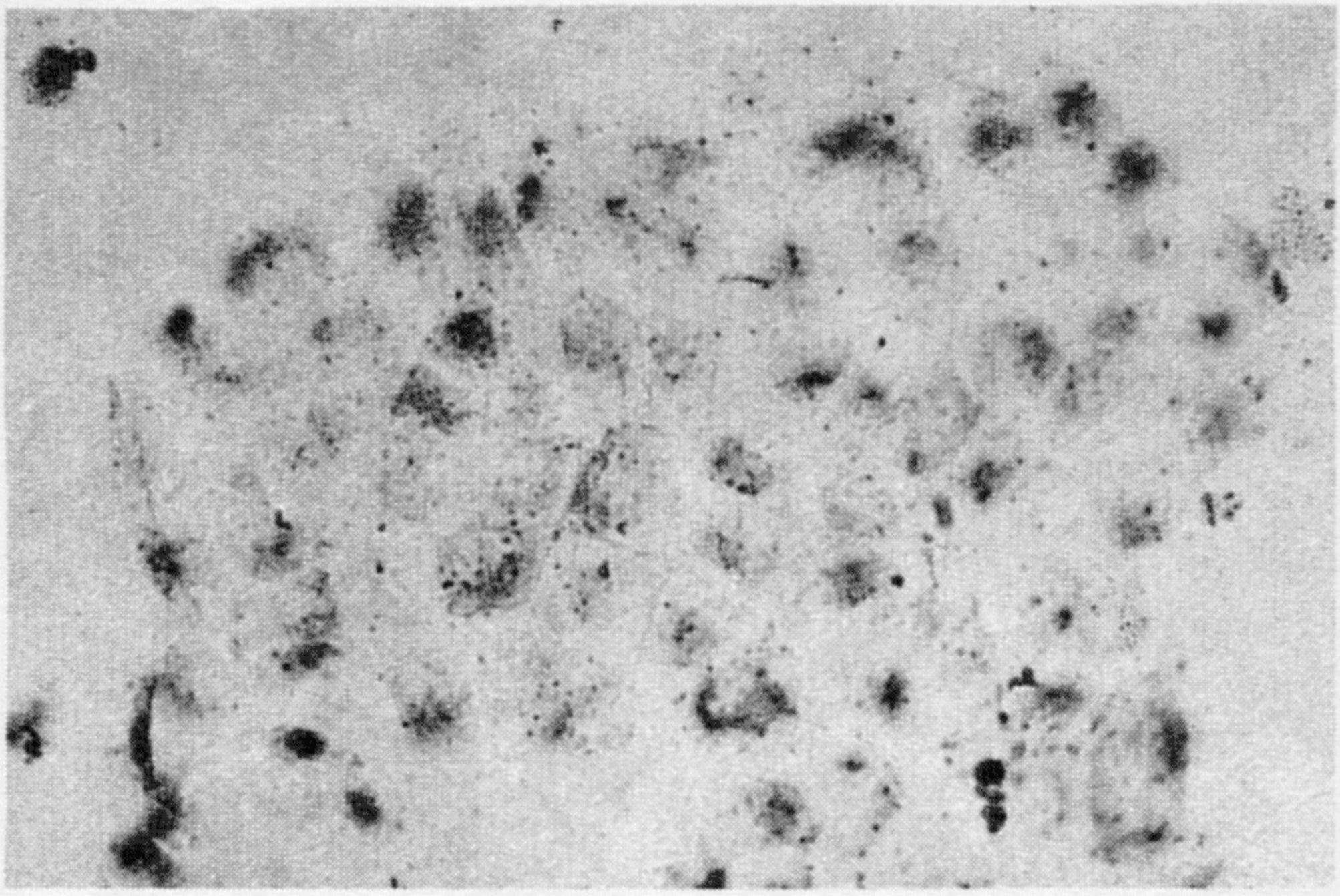

Figure 14.

Control PK cell slide culture 5 hrs after sham inoculation, acid phosphatase (alpha-naphtyl phosphate, hexazo-p-rosanilin), (200x).

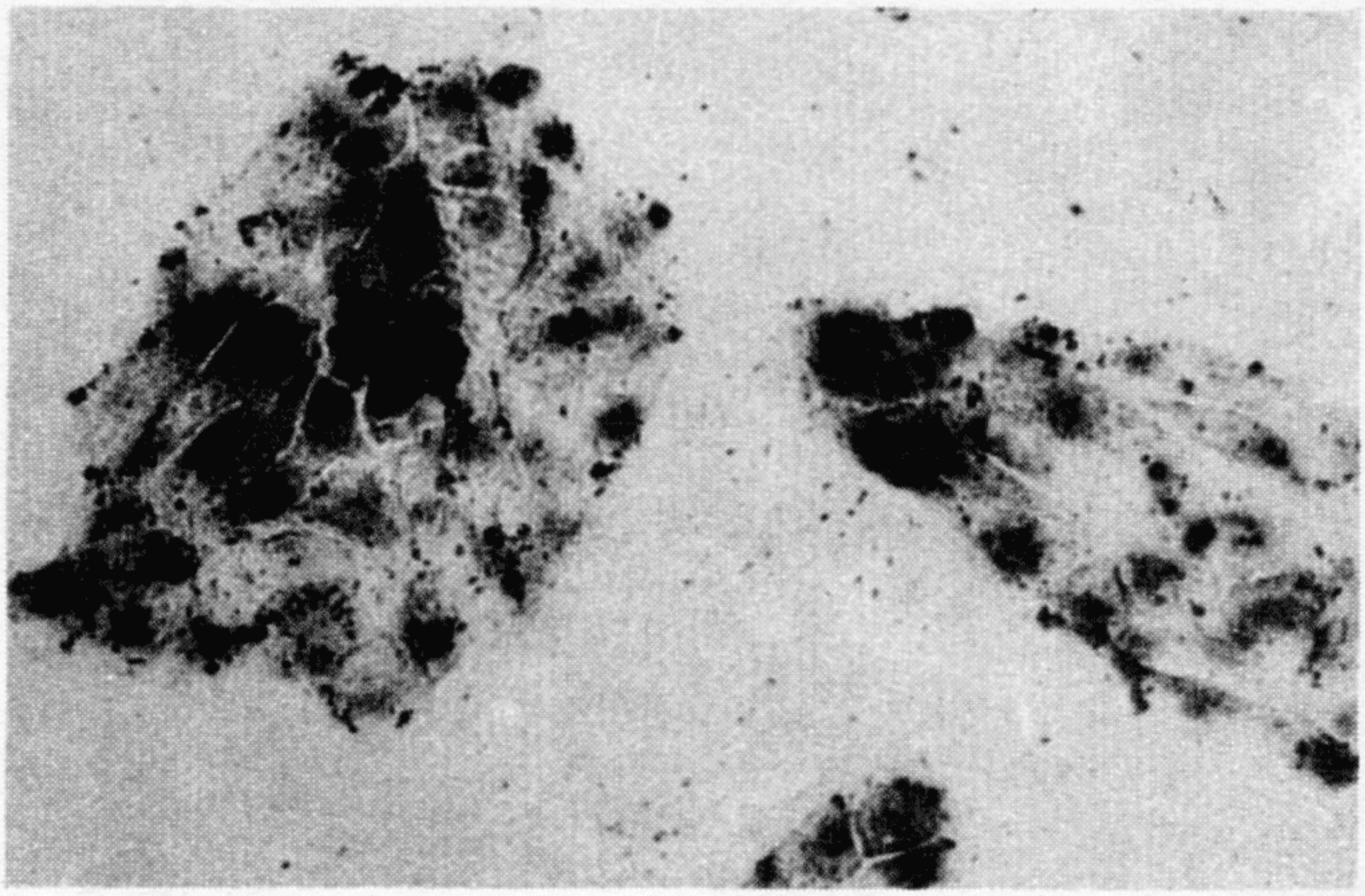

Figure 15.

TDV-infected PK cells 5 hrs after inoculation, acid phosphatase (alpha-naphtyl phosphate,hexazo-p-rosanilin), (200x).