

Continued differentiation of mammalian embryos in utero after blockage of implantation

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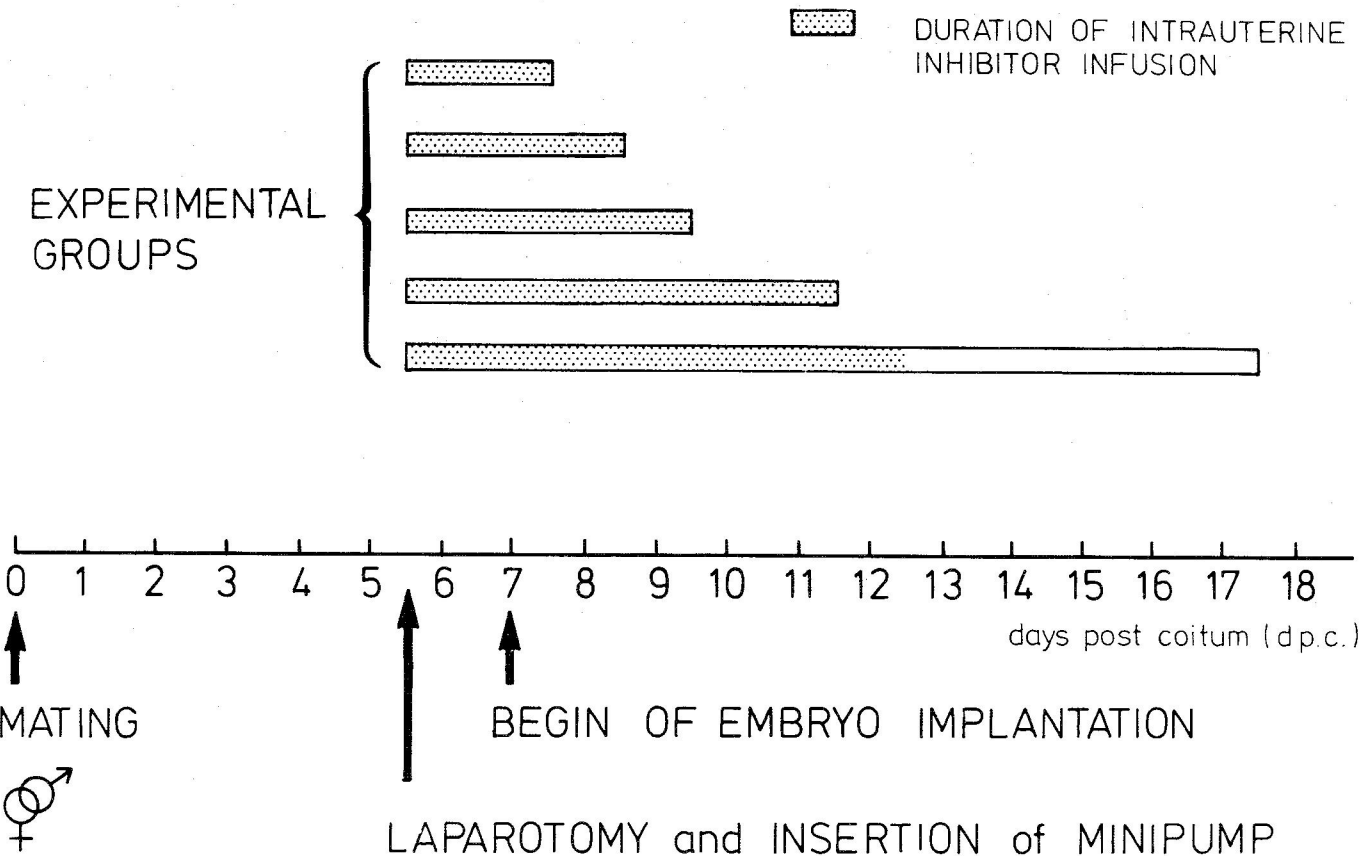
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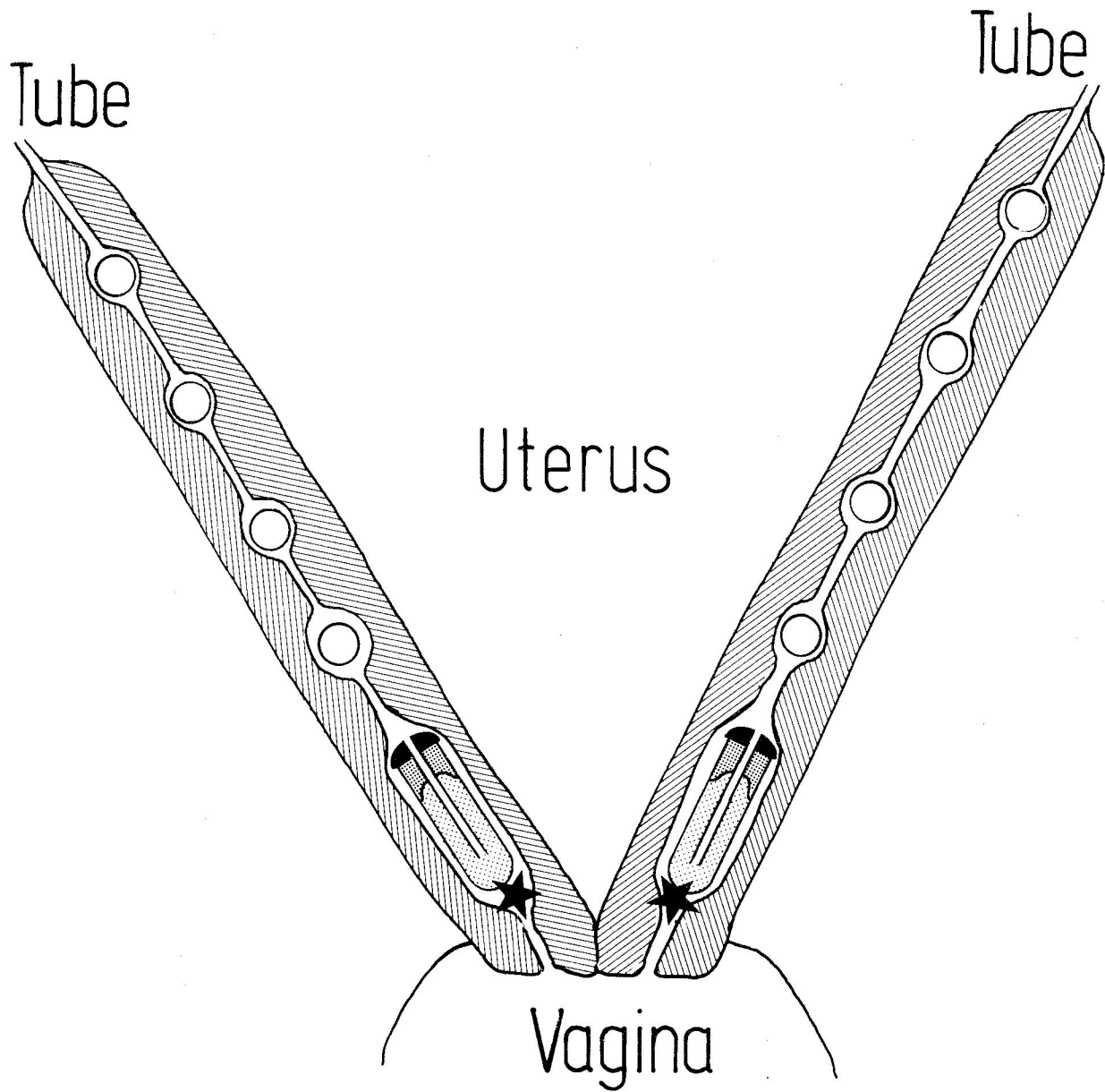
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(Abstract No. 22)

PREVIOUS OBSERVATIONS TEND TO SUGGEST THAT THE DEVELOPMENT OF MAMMALIAN EMBRYOS BEYOND STAGES EQUIVALENT TO IMPLANTATION DEPENDS SOMEHOW ON THE FORMATION OF AN INTIMATE CELLULAR CONTACT OF THE TROPHOBLAST WITH UTERINE TISSUES. IN VITRO CULTURE GIVES SATISFACTORY RESULTS ONLY UP TO THE LATE BLASTOCYST STAGE, IF ATTACHMENT IS IMPOSSIBLE; FURTHER DEVELOPMENT CAN BE OBTAINED IF THE CULTURE IS STARTED WITH POSTIMPLANTATION STAGES.

IN A SERIES OF EXPERIMENTS ON INTERFERENCE WITH IMPLANTATION BY PROTEINASE INHIBITOR ADMINISTRATION IN VIVO, WE USED CONTINUOUS INTRAUTERINE INFUSION OF APROTININ (TRASYLOL) BY OSMOTIC MINIPUMPS. THIS TREATMENT EFFECTIVELY INHIBITS BLASTOLEMMASE ACTIVITY WHICH IS ESSENTIAL FOR DISSOLUTION OF THE EXTRACELLULAR BLASTOCYST COVERINGS IN THE BEGINNING OF THE IMPLANTATION PROCESS. AS A RESULT, THESE COVERINGS REMAIN INTERPOSED BETWEEN TROPHOBLAST AND UTERINE EPITHELIUM THEREBY PREVENTING CONTACT FORMATION. WHILE THE MAJORITY OF THE EMBRYOS MANAGES TO HATCH MECHANICALLY FROM THE COVERINGS SO THAT PARTIAL ATTACHMENT IS POSSIBLE, 25% OF THEM WERE FOUND COMPLETELY ENCASED IN THE COVERINGS AT 9 1/2 DAYS POST COITUM, I.E. 2 1/2 DAYS AFTER IMPLANTATION WOULD NORMALLY HAVE STARTED. THESE EMBRYOS ARE STILL COMPLETELY FREE IN THE UTERINE LUMEN, BUT, INTERESTINGLY, DIFFERENTIATION IS NOT HAMPERED OR ONLY SLIGHTLY RETARDED. THEREFORE A CONDITION IS FOUND, IN THIS EUTHERIAN MAMMAL, WHICH WAS PREVIOUSLY ONLY SEEN IN MARSUPIALS, I.E. A FREE-LIVING CONCEPTUS IN THE UTERINE LUMEN WITH NEURAL TUBE, SOMITES ETC. IN CONTRAST TO THE REGULAR DEVELOPMENT OF THE EMBRYO PROPER, THE TROPHOBLAST WAS FOUND TO UNDERGO EARLY DEGENERATION FROM WHICH IT WAS RESCUED ONLY IN CASES WHERE IT MANAGED TO CONTACT MATERNAL TISSUE.





OSMOTIC MINIPUMPS (ALZA CORP., MODEL No. 1701) WERE PLACED IN THE UTERINE LUMEN NEAR THE CERVIX, DURING LAPAROTOMY PERFORMED AT 5 1/2 DAYS POST COITUM (D P.C.). THE STARS INDICATE THE OPENINGS IN THE ANTIMESOMETRIAL UTERINE WALL THROUGH WHICH THE MINIPUMPS WERE INSERTED AND WHICH WERE CLOSED THEREAFTER. ONE UTERUS ALWAYS RECEIVED A MINIPUMP LOADED WITH APROTININ (TRASYLOL^R) (17 OR 85 MG/ML) WHILE THE OTHER SIDE SERVED AS A CONTROL (MINIPUMP LOADED WITH VEHICLE FLUID). PUMPING RATE 1 μ L/H FOR 7 DAYS.

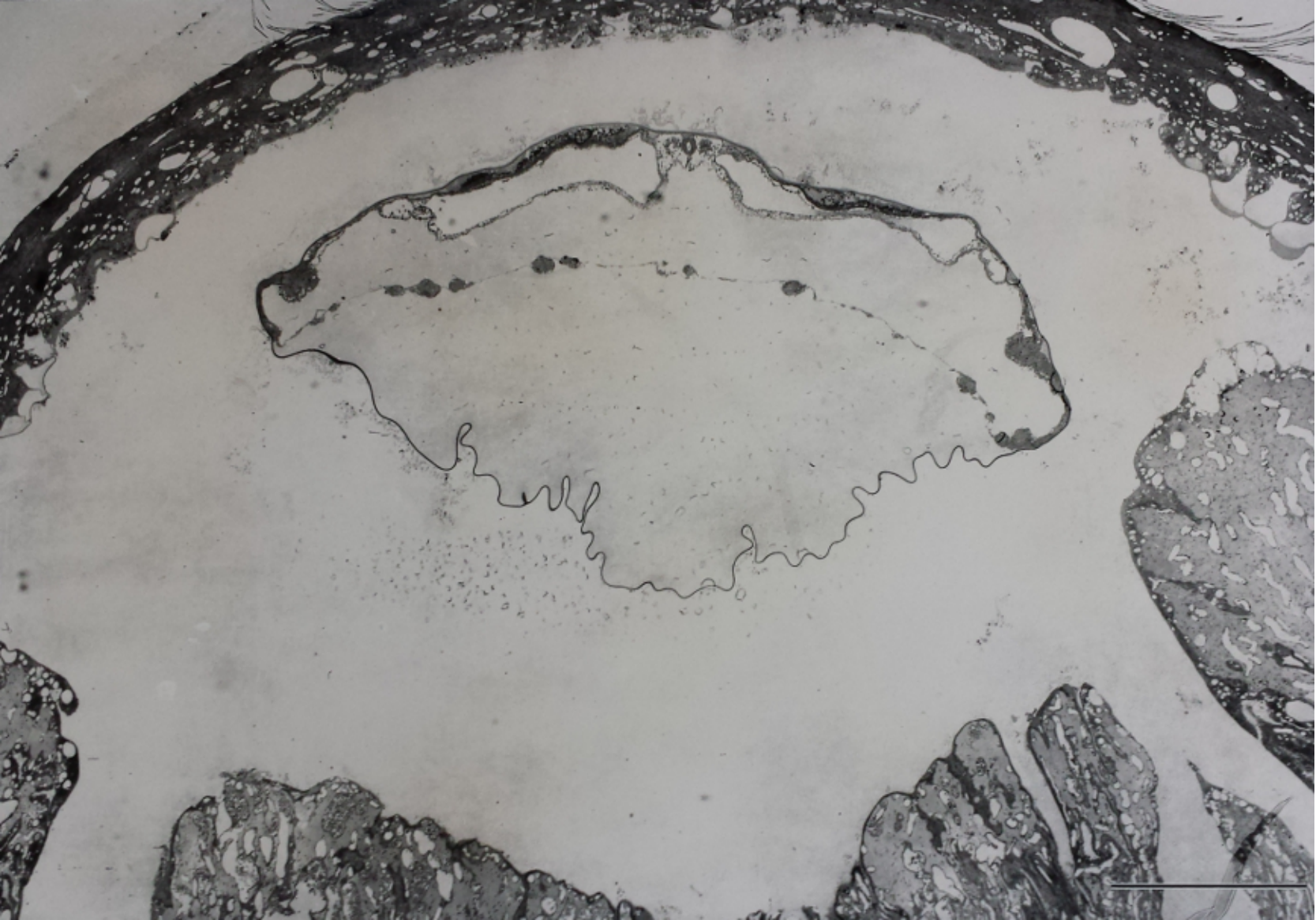


FIG. 1. THE DISSOLUTION OF BLASTOCYST COVERINGS WAS BLOCKED BY CONSTANT INTRA-UTERINE INFUSION OF THE PROTEINASE INHIBITOR, APROTININ. AS A RESULT, THIS BLASTOCYST LIES STILL COMPLETELY FREE IN THE UTERINE LUMEN AT 9 1/2 D P.C., I.E. 2 1/2 DAYS AFTER IMPLANTATION HAS STARTED IN THE CONTROLS. SPACES BETWEEN THE SURFACE OF THE BLASTOCYST AND THE ENDOMETRIUM ARE EXAGGERATED DUE TO SHRINKAGE DURING DEHYDRATION AND ARALDITE EMBEDDING. ORIENTATION OF THE BLASTOCYST WITHIN THE UTERUS IS ABNORMAL, ITS EMBRYONIC POLE FACING THE ANTIMESOMETRIAL (ABOVE) RATHER THAN THE MESOMETRIAL PART OF THE ENDOMETRIUM. SEMITHIN SECTION, BAR = 1 MM. (COMPARE WITH FIG. 6).



FIG. 2. DETAILS OF THE ANLAGE OF THE EMBRYO PROPER FROM THE SITE SHOWN IN FIG. 1. SLIGHT RETARDATION OF DEVELOPMENT AND A MODERATE HYPOPLASIA CAN BE NOTED. NEVERTHELESS, A REMARKABLE DEGREE OF DIFFERENTIATION HAS BEEN REACHED IN SPITE OF THE BLOCKAGE OF TROPHOBLAST ATTACHMENT (NEURAL TUBE, SOMITES, DORSAL AORTAE ETC.). CONNECTION WITH THE YOLK SAC (FORMER BLASTOCYST CAVITY, BELOW) IS STILL WIDE. BAR = 100 μ m. (COMPARE WITH FIG. 7).

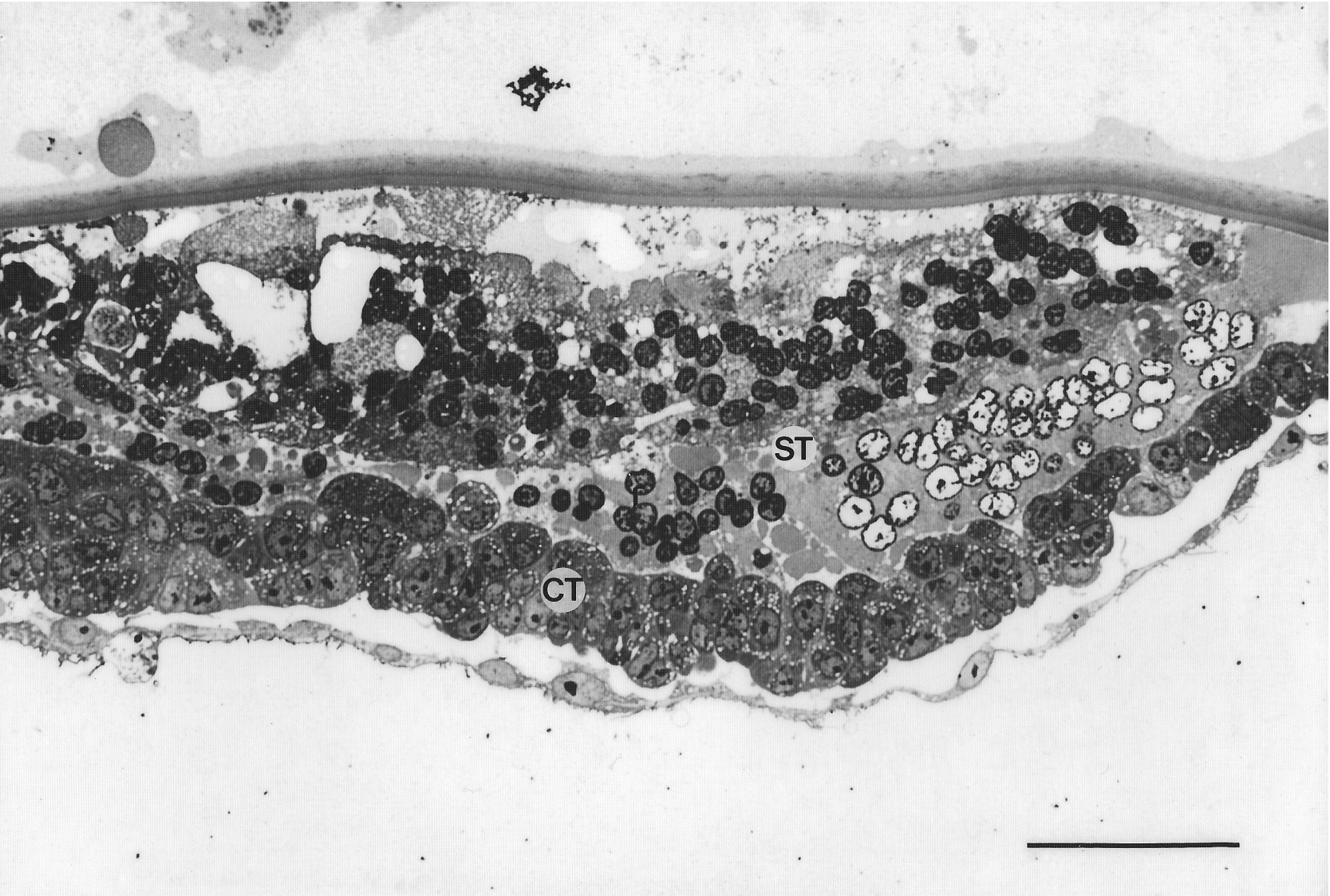


FIG. 3. DETAIL OF THE TROPHOBLAST NEXT TO THE EMBRYONIC ANLAGE OF THE SITE SHOWN IN FIG. 1. THIS PART OF THE TROPHOBLAST IS NORMALLY HIGHLY INVASIVE AND INVOLVED IN THE FORMATION OF THE CHORIOALLANTOIC PLACENTA. IN THIS CASE IT HAS NOT BEEN ABLE TO ESTABLISH CONTACT WITH THE ENDOMETRIUM SINCE THE NON-DISSOLVED BLASTOCYST COVERINGS (ABOVE) REMAIN INTERPOSED. NOTICE THE THREE LAYERS OF THE BLASTOCYST COVERINGS (GLOIOLEMA, MUCOPROTEIN LAYER, NEOZONA). THE SYNCYTIOTROPHOBLAST (ST) SHOWS SIGNS OF DEGENERATION, APPARENTLY PROGRESSING IN SEVERAL WAVES. THE CYTOTROPHOBLAST (CT) APPEARS HEALTHY. BAR = 50 μ m. (COMPARE WITH FIG. 8).

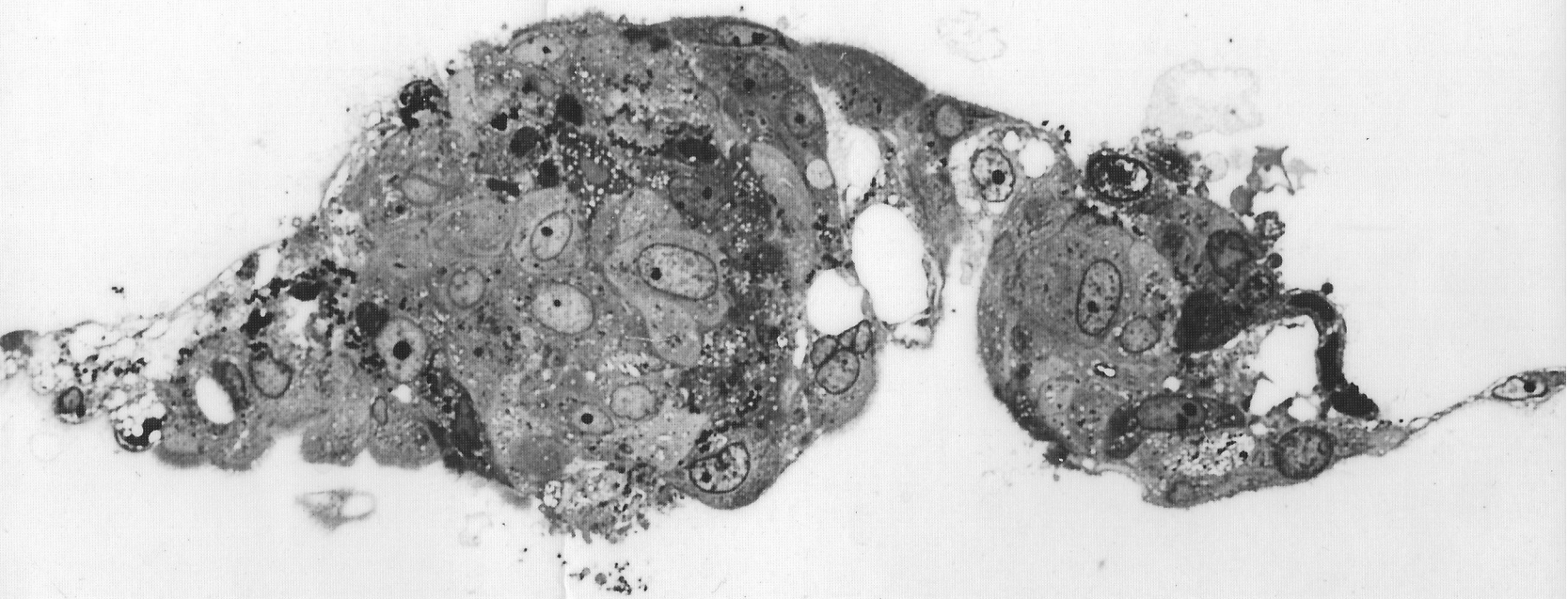


FIG. 4. UNATTACHED TROPHOBLASTIC KNOBS OF THE SITE SHOWN IN FIG. 1. TROPHOBLASTIC KNOBS ARE THE INVASIVE ELEMENTS IN THE ABEMBRYONIC HEMISPHERE OF A BLASTOCYST. IN THIS CASE THEY ARE CONSIDERABLY ENLARGED BUT FIRST SIGNS OF INCIPIENT DEGENERATION ARE RECOGNIZABLE. BAR = 50 μ m. (COMPARE WITH FIG. 9).

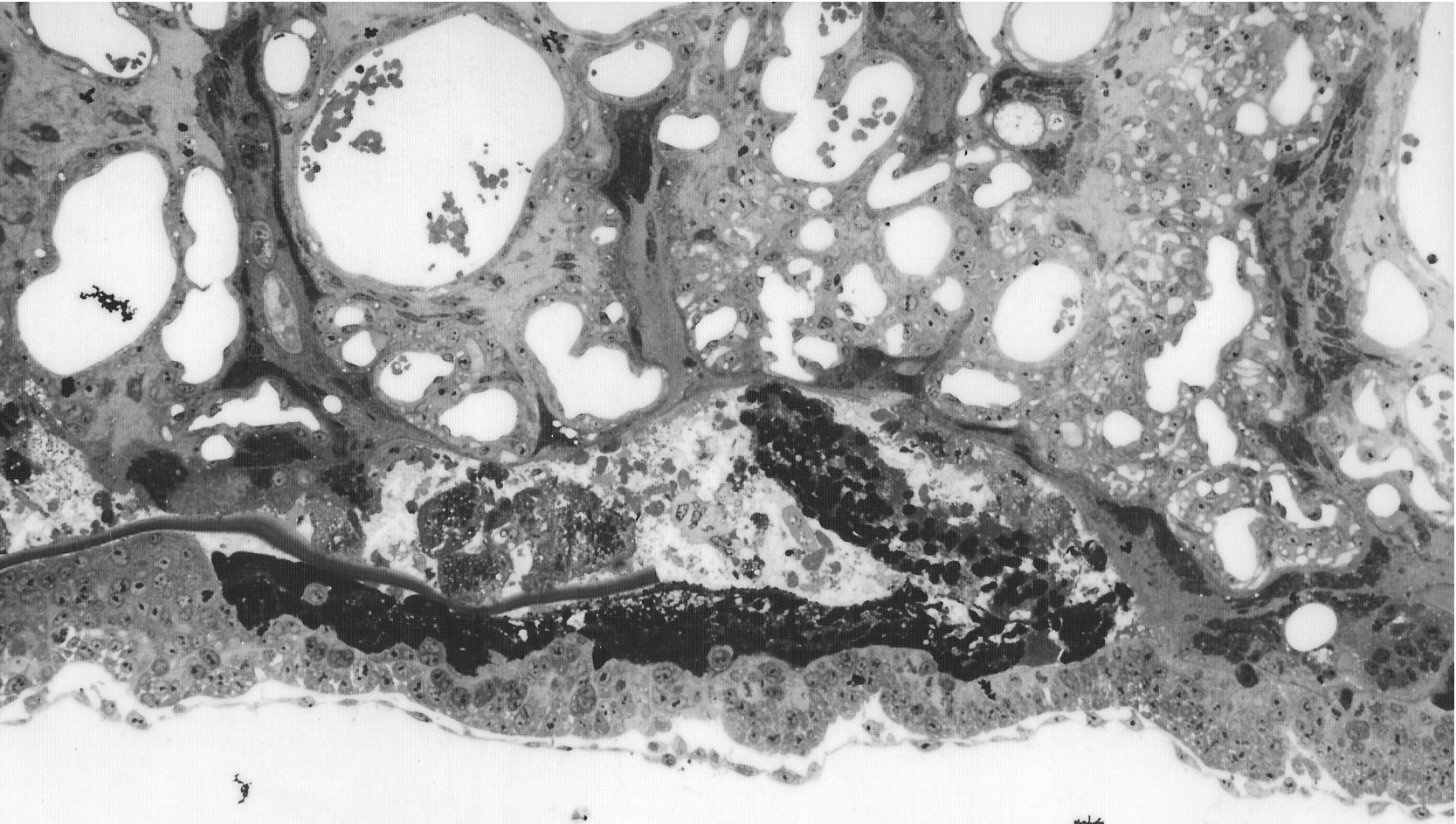


FIG. 5. THE SYNCYTIOTROPHOBLAST SEEMS TO BE PROGRAMMED FOR RAPID DEGENERATION BUT IS NORMALLY RESCUED (UNTIL THE END OF PREGNANCY) IF IT ESTABLISHES DIRECT CELLULAR CONTACT WITH MATERNAL TISSUES. DETAIL FROM ANOTHER IMPLANTATION SITE OF AN APROTININ-TREATED UTERUS. DISSOLUTION OF THE BLASTOCYST COVERINGS WAS BLOCKED, BUT IN CONTRAST TO FIG. 1 - 4, THIS BLASTOCYST HAS HATCHED MECHANICALLY BY RUPTURING THE COVERINGS. AT THE LEFT SIDE OF THE PICTURE, A FRAGMENT OF THE BLASTOCYST COVERINGS (SHARP EDGE INDICATING ABSENCE OF BLASTOLEMMASE ACTIVITY) AND DETRITUS ARE INTERPOSED BETWEEN THE TROPHOBLAST AND THE ENDOMETRIUM. HERE, THE SYNCYTIOTROPHOBLAST DEGENERATES (DARK MASSES WITH PYCNOTIC NUCLEI, SEE ALSO FIG. 3) WHILE THE CYTOTROPHOBLAST LOOKS HEALTHY. AT RIGHT, CONTACT WITH THE MATERNAL TISSUES WAS FORMED AND HERE THE SYNCYTIOTROPHOBLAST IS INTACT AND INVADES THE ENDOMETRIUM. BAR = 100 μ m. (COMPARE WITH FIG. 3 AND 8).

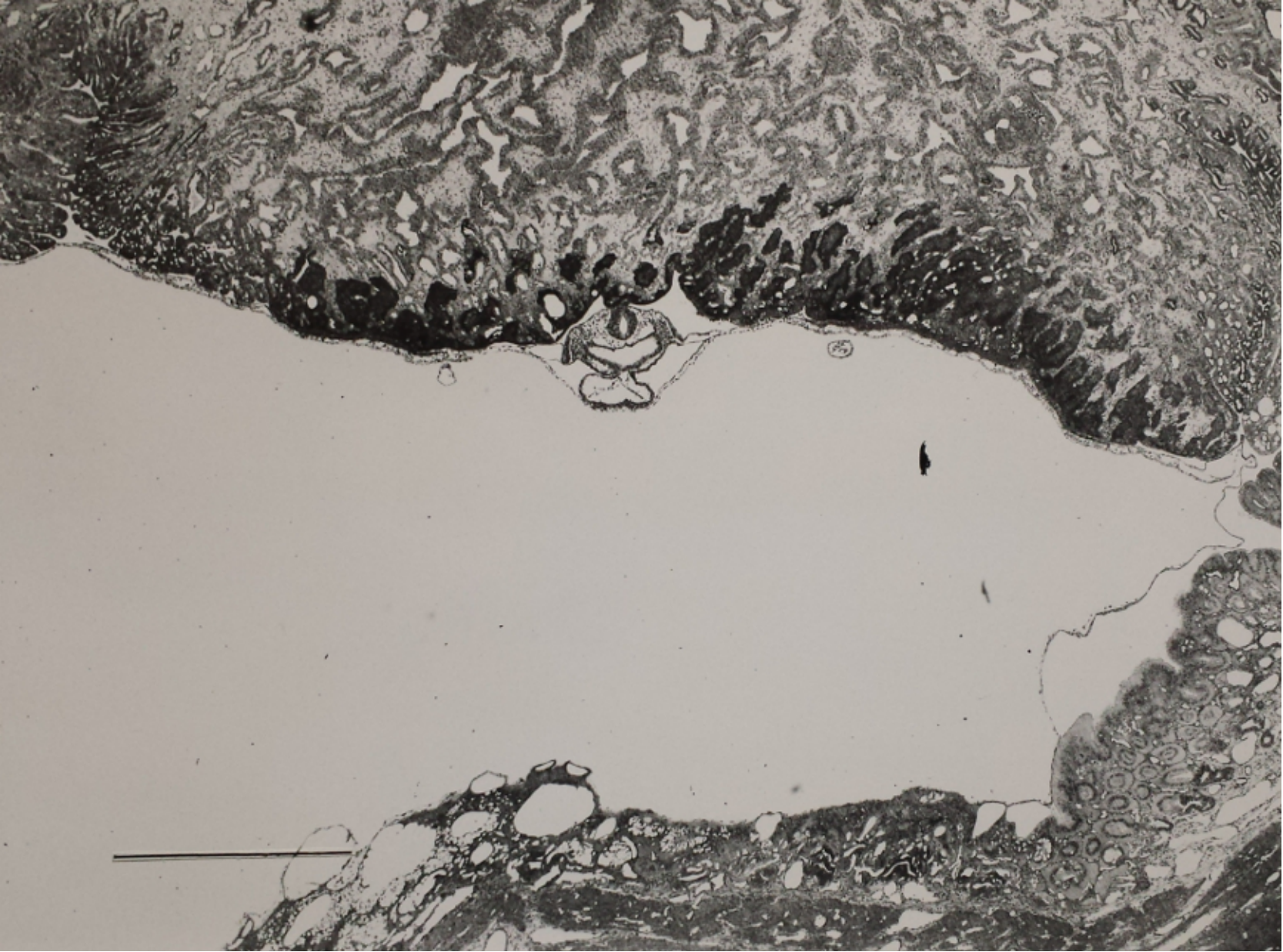


FIG. 6. NORMAL IMPLANTATION IS SHOWN HERE FOR COMPARISON. SAME STAGE AS FIG. 1 - 5 (9 1/2 D P.C.). CORRECT ORIENTATION IN THE UTERUS, WITH THE EMBRYONIC POLE FACING THE MESOMETRIAL ENDOMETRIUM (ABOVE) AND THE ABEMBRYONIC POLE LOCATED ANTIMESOMETRIALLY (BELOW). THE EXTRACELLULAR BLASTOCYST COVERINGS HAVE BEEN DISPOSED OF. THE TROPHOBLAST HAS MADE CONTACT WITH THE ENDOMETRIUM, FIRST (FROM 7 D P.C. ON) IN THE ABEMBRYONIC-ANTIMESOMETRIAL REGION (OBPLACENTA, YOLK SAC PLACENTA) AND THEREAFTER (FROM 8 D P.C. ON) IN THE EMBRYONIC-MESOMETRIAL AREA ON BOTH SIDES OF THE ANLAGE OF THE EMBRYO PROPER (DEFINITIVE, CHORIOALLANTOIC PLACENTA). AT THE STAGE SHOWN HERE, THE EPHEMERAL YOLK SAC PLACENTA ALREADY SHOWS THE FIRST SIGNS OF DEGENERATION. BAR = 1 MM. (COMPARE WITH FIG. 1).

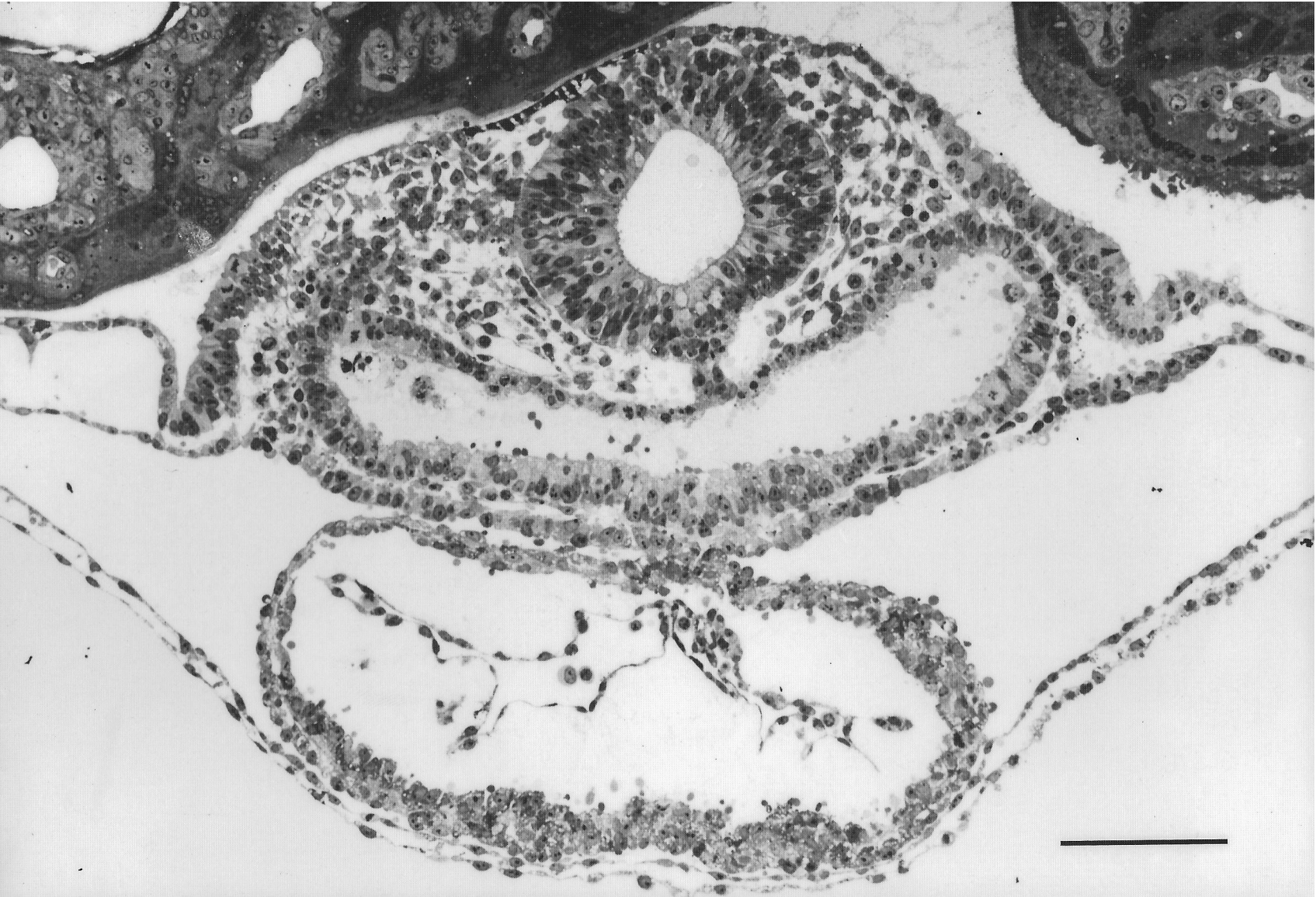
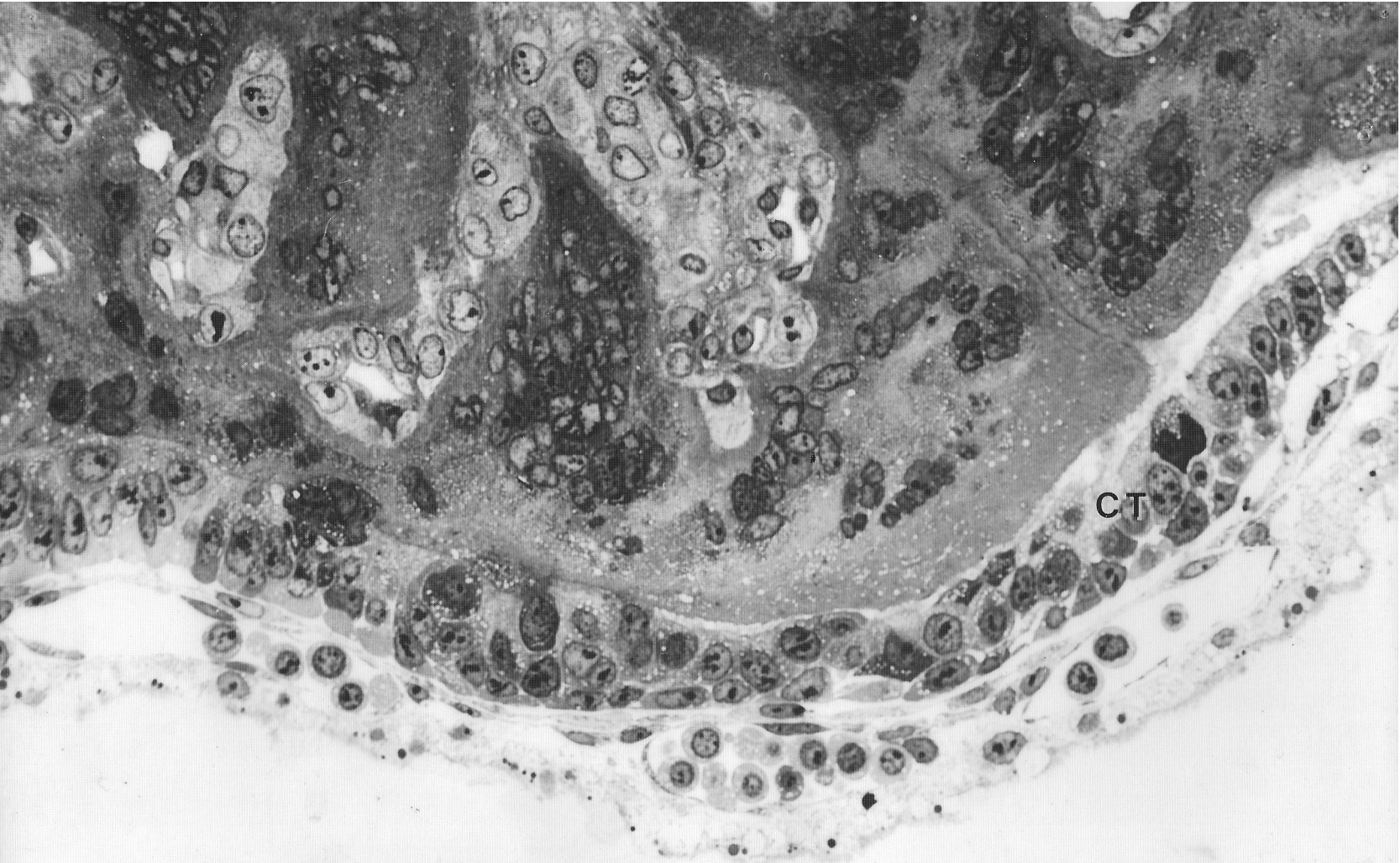


FIG. 7. THE EMBRYONIC ANLAGE OF FIG. 6 IS SLIGHTLY FURTHER DEVELOPED THAN THE NON-IMPLANTED EMBRYO SHOWN IN FIG. 2. THE SOMITES HAVE ALREADY DISINTEGRATED. THIS SECTION PASSES THROUGH THE HEART ANLAGE NOT MET WITH IN FIG. 2. BAR = 100 μ m.



CT



FIG. 8. DETAIL FROM THE MESOMETRIAL INVASION ZONE (FORMATION OF THE CHORIOALLANTOIC PLACENTA) OF THE CONCEPTUS SHOWN IN FIG. 6. NEXT TO THE ANLAGE OF THE EMBRYO PROPER, THE CYTOTROPHOBLAST (CT) HAS PROLIFERATED AND HAS BECOME MULTI-LAYERED. IN THE OUTER LAYER, CELLS FUSE WITH EACH OTHER AND FORM THE SYNCYTIOTROPHOBLAST (LARGE, INTENSELY STAINED NUCLEI) WHICH IN TURN FUSES WITH THE UTERINE EPITHELIAL SYNCYTIUM AND INVADES THE ENDOMETRIUM. UNDERNEATH THE TROPHOBLAST: EXTRA-EMBRYONIC MESODERM (WITH BLOOD VESSELS) AND ENDODERM. BAR = 50 μ m. (COMPARE WITH FIG. 3).

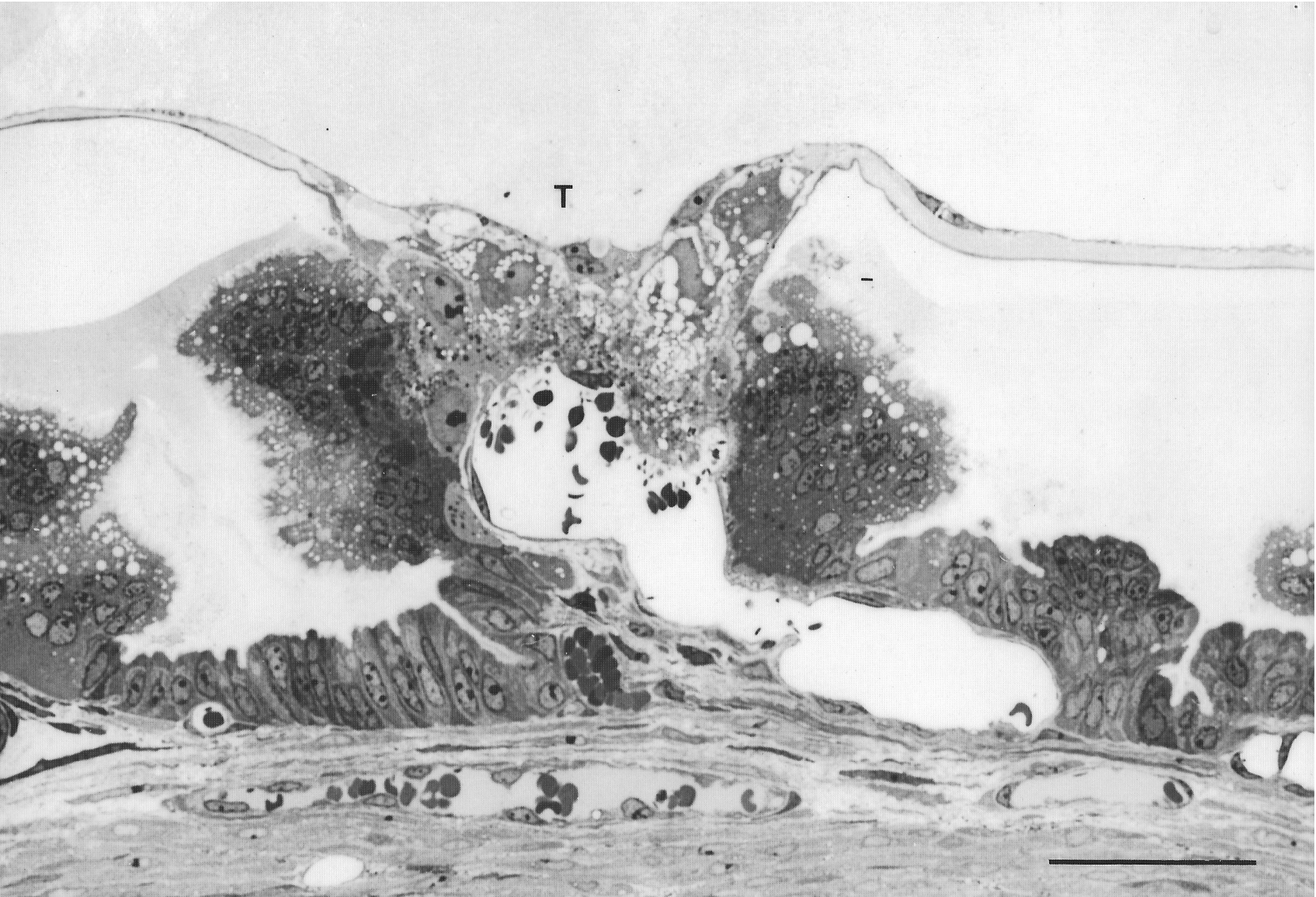


FIG. 9. TROPHOBLASTIC KNOBS ARE THE INVASIVE ELEMENTS IN THE ABEMBRYONIC HEMISPHERE WHERE THE YOLK SAC PLACENTA IS BEING FORMED. AFTER THE BLASTOCYST COVERINGS HAVE BEEN DISSOLVED, THE TROPHOBLASTIC KNOBS CONTACT AND INVADE THE ENDOMETRIUM. THE PEAK OF THIS PROCESS IS REACHED ALREADY ONE DAY BEFORE MESOMETRIAL IMPLANTATION STARTS; THEREFORE, WE ARE SHOWING HERE THE 8 1/2 D P.C. STAGE (RATHER THAN 9 1/2 D P.C. AS IN THE PREVIOUS FIGS.). THE TROPHOBLASTIC KNOB (T) HAS PENETRATED THROUGH THE UTERINE EPITHELIUM AND HAS ERODED A MATERNAL BLOOD VESSEL WHERE IT CAUSED FORMATION OF A MARGINAL THROMBUS. BAR = 50 μ m. (COMPARE WITH FIG. 4).