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<b>13. ABSTRACT (Maximum 200 Words)</b>  Our goal is to develop a new in vitro model of human prostate cancer. Available models do not faithfully replicate prostate cancer in situ and therefore activities of experimental drugs in these models do not accurately predict clinical efficacy. We are developing "tissue slice culture". Extremely thin slices of fresh tissue are prepared and placed into culture. If conditions are optimal, these slices retain their appropriate structure and functions, providing a realistic model to screen drugs. The tasks were to optimize preparation of slices, develop optimal culture conditions for maintenance, test the validity of these cultures as an accurate in vitro model, and initiate pilot studies to screen candidate therapeutic agents. During this first year, we focused on preparation of slices. This proved challenging due to the unique constituency of prostate tissue, but we obtained tissue cores that we could slice and place into culture. We tested several media and chose one that supported the best maintenance of structure and function. Immunohistochemical protocols were developed to evaluate function of diverse cell types in the tissue. Recently, we tested an experimental compound, vitamin D, for its effects on prostate tissue slices and these slices are ready for evaluation.				
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## INTRODUCTION

At the present time, there is no experimental model system that accurately predicts the clinical efficacy of a drug against prostate cancer. Monolayer cell cultures, co-cultures combining stromal and epithelial cells, three-dimensional cultures in matrix supports, and xenografts are currently used to evaluate pre-clinical activity of experimental compounds. Numerous agents that kill or inhibit the growth of prostate cancer cells in one or more of these model systems have been identified, yet an effective chemotherapeutic agent for prostate cancer is still not available. A more realistic model of prostate cancer is urgently needed, and we suggest that tissue slice cultures will fill this need.

Tissue slices have been used for biochemical studies for several decades. Although typically viable for only a short period of time, tissue slices from liver, kidney, heart and lung were nevertheless used for many diverse studies, including toxicology, pharmacology, organ preservation and metabolism. In the early 1990's, methodology was developed for long term maintenance of tissue slices. However, the recent advancement that promises to extend the use of tissue slice cultures to a wide variety of organs and applications is precision cutting. This is accomplished with an automated microtome (the Krumdieck tissue slicer), which permits the rapid preparation of aseptic slices of live tissue with nearly identical dimensions. This instrument eliminates the major source of error in tissue slice work, namely, the use of slices of different and uneven thickness with irregular, non-reproducible damage at the cut surfaces. This improvement eliminates the variable of gas and nutrient exchange by diffusion that exists among unequally or unevenly cut slices.

With seed funds from a CaP CURE prostate cancer award, we purchased the Krumdieck tissue slicer and performed pilot studies to begin to master the technical challenges of prostate tissue slice culture. We used a special core borer to obtain tissue from a radical prostatectomy specimen. This core of fresh tissue was mounted on the Krumdieck microtome and thin sections were cut by a rapidly reciprocating, disposable microtome blade. Throughout the cutting procedure, the tissue remains immersed in chilled physiologic fluid. Upon cutting, the thin sections gently shoot into a reservoir of physiologic fluid, and are then transferred to grids in culture vessels with the aid of a tool that resembles a miniaturized minnow scoop. The tissue slices are rotated in a specially designed apparatus that eliminates conventional mechanical shaking, which inevitably leads to disintegration of tissue at the slice-medium interface. In our pilot study, we were able to obtain cores of fresh tissue, cut thin sections and successfully transfer the sections to culture vessels. An additional challenge was to recover the thin sections for analysis after culture. We were able to fix the thin sections, transfer them to embedding cassettes, dehydrate and embed in paraffin, and cut 5  $\mu\text{m}$  sections for histological analysis after staining with Hematoxylin and Eosin (H & E). We also performed immunohistochemical analyses to check maintenance of function.

Our proposed plan is to improve our technical abilities to prepare, process and evaluate tissue slice cultures and to develop optimal culture conditions for these tissues *in vitro*. Our long term objective is to use tissue slice cultures as a pre-clinical model system to screen therapeutic agents.

## BODY

Our **first designated task was to optimize preparation of prostatic tissue slices.** We have made progress towards this goal. For optimal tissue slice culture, it is important to obtain uniform tissue cores from surgical specimens. To facilitate this process, we purchased an automated tissue coring press to replace our hand-held, manual tissue coring tool. With the free-hand method, we had found it difficult to reproducibly obtain tissue cores of consistent and uniform diameter. The raggedy nature of such cores was detrimental to proper attachment to the tissue holder and subsequent sectioning. While the automated corer has been an improvement, we still have difficulty in obtaining optimal cores from every surgical specimen. This appears to be due to the particular constituency of prostate tissue, and to the variability and heterogeneity of constituency among specimens, presumably related to histological make-up of the specimens. For example, some tissues are quite soft while others are fibrous. One unexpected problem has been that the corer becomes dull after only a few uses, presumably due to fibrous tissue. This necessitates precision sharpening at the manufacturer in Alabama, causing additional expense and delays.

Nevertheless, we have been able to obtain adequate cores from several prostatectomy specimens and these were successfully sliced and placed into culture. Another goal of task 1 was to optimize the fixation and embedding protocol for tissue slice cultures. We have not encountered too much difficulty here and are able to retrieve the slices after culture, fix, embed in paraffin, and cut at 5  $\mu\text{m}$  for subsequent histologic and immunohistochemical analysis. The one problem that we have encountered is that we lose a fair amount of the thin tissue slice as we trim the face of the block. Our idea to prevent this is to lay the tissue slice on a piece of nitrocellulose filter paper as it is embedded in paraffin in the cassette. The filter paper can then serve as a "marker" on which to trim the block, thereby preserving the limited amount of tissue in each block.

The **second task was to develop optimal culture conditions for the maintenance of tissue structure and function.** We have also made progress toward this goal. In our first comparative study of different culture media, we evaluated two media that we routinely use to establish and maintain primary cultures of human prostatic epithelial cells. These two media, "Complete PFMR-4A" and "Complete MCDB 105" are both serum-free and consist of defined basal media (PFMR-4A and MCDB 105, respectively) supplemented with cholera toxin, epidermal growth factor, insulin, phosphoethanolamine, hydrocortisone, selenium, alpha-tocopherol, retinoic acid, bovine pituitary extract, and gentamicin. Tissue slices were retrieved and fixed after culture in the two media for 24 and 48 hr. Histological analysis of H & E-stained sections (Figure 1) and immunohistochemical staining with antibodies specific for basal epithelial cells (cytokeratin 5) (Figure 2), secretory epithelial cells (prostate-specific antigen and cytokeratin 18, Figure 2), and smooth muscle cells (smooth muscle  $\alpha$ -actin, Figure 3) suggested that Complete PFMR-4A maintained structural and functional integrity better than Complete 105. Therefore, Complete PFMR-4A was chosen for future studies.

In the next study, Complete PFMR-4A was supplemented with or without 10 nM of the synthetic androgen, R1881. Androgen is believed to be necessary for the maintenance of differentiated prostatic secretory epithelial cells, and indeed, histologic and immunochemical analyses of tissue slices maintained for 24 and 48 hours suggested that the secretory epithelium was better

maintained in the presence of androgen (Figures 4, 5 and 6). Therefore, Complete PFMR-4A with 10 nM of R1881 was chosen for subsequent studies.

We next performed a time course study to determine how long structure and function could be maintained in Complete PFMR-4A with 10 nM of R1881. Tissue slices were cultured for 24, 48 and 72 hours and histology and antigen expression were compared to uncultured tissue. Structure and function after 24 hours of culture were comparable to that of uncultured tissue, but degeneration of the secretory epithelium began to be apparent at 48 hours and was quite advanced by 72 hours. We concluded that additional optimization of the culture environment will be necessary for long-term maintenance of tissue slice cultures, and this will be a goal in the coming year. In this time course study, we also began to expand the number of immunohistochemical markers that we will use to assess function of different cell types in the tissues. In addition to the classic basal epithelial cell marker of cytokeratin 5, p63 will be evaluated. We used monolayer cultures to optimize antibody concentration, and we will evaluate p63 expression in these sections. The proliferation marker Ki-67 was optimized in monolayer cultures and will also be evaluated to monitor proliferation in tissue slice cultures.

Since tissue structure and function appeared to be fairly well-maintained after 24 hours of culture in Complete PFMR-4A with R1881, we decided to **initiate pilot studies to use tissue slice cultures to screen candidate therapeutic agents for prostate cancer (task 4)**. We chose the active metabolite of vitamin D,  $1\alpha$ -dihydroxyvitamin D<sub>3</sub> (1,25D) as our first experimental agent. This compound was chosen because 1,25D has emerged as a leading candidate to prevent or treat prostate cancer based on epidemiologic studies, tumor suppressor activity in *in vitro* and *in vivo* models of prostate cancer, and clinical trials. Our experience with the activity of 1,25D in monolayer cultures of prostatic epithelial and stromal cells is extensive so 1,25D seemed an appropriate choice for our first pilot study with tissue slice cultures.

Tissue slices were prepared and cultured for 24 hours in Complete PFMR-4A with 10 nM of R1881 and +/- 50 nM 1,25D. These slices were retrieved, fixed, embedded, and sectioned at 5  $\mu$ m. Preliminary results are shown in Figure 7, but additional histological and immunohistochemical analysis will be done in the coming year to assess the effects of 1,25D. Expected effects of 1,25D would include reduction of basal epithelial cell proliferation, increased differentiation of the secretory epithelium, and perhaps apoptosis of cancer cells. A known gene target of 1,25D, vitamin D 24-hydroxylase (CYP 24), would be expected to be significantly induced in prostatic epithelial cells after 24 hours of treatment.

#### **KEY RESEARCH ACCOMPLISHMENTS**

- Improved method of reproducibly obtaining tissue cores from prostatectomy specimens
- Selected an optimal basal medium that supports maintenance of tissue structure and function for 24 to 48 hours
- Determined that androgen is required for optimal maintenance of differentiated secretory cells in tissue slice cultures
- Developed protocols to use additional antibodies for evaluation of cellular function in tissue slice cultures

- Initiated pilot studies to evaluate the activity of an experimental chemotherapeutic agent, 1,25D, in tissue slice cultures

## **REPORTABLE OUTCOMES**

None.

## **CONCLUSIONS**

Our progress is good but technical challenges have been greater than anticipated, seemingly due to the unique nature of prostate tissue (in comparison to “soft” tissues such as brain and liver, for instance, which have been successfully used in tissue slice cultures for many years). We will continue to work on obtaining uniform cores, and our next goal will be to obtain slices for which the precise thickness can be known. To this end, we have purchased a tissue slice thickness gauge, but we have not been able to consistently use it because cores are still not completely optimal. It will be important to evaluate the variable of tissue thickness (50 to 300  $\mu\text{m}$ ) for its effects on maintenance of structure and function in our future studies.

While we continue to perform elements of task 1 (optimizing preparation of tissue slices), we have already achieved several goals of task 2 (to develop optimal culture conditions). After comparative analyses, we chose Complete PFMR-4A as the standard medium for future studies. We also determined that androgen was required for optimal maintenance of tissue slices, so our standard medium formulation will also include 10 nM of R1881. As we expand our repertoire of immunohistochemical markers to evaluate function of diverse cell types in the tissue slices, we will begin this year to systematically vary the growth factors in the serum-free Complete PFMR-4A and evaluate effects on structure and function.

While the goal of task 4, to initiate pilot studies to use tissue slice cultures to screen candidate therapeutic agents for prostate cancer, cannot be fully approached until we further optimize the tissue slice cultures, we initiated a pilot study with a well-studied experimental agent, 1,25D. The activity of 1,25D on prostate cells has been extensively documented in prostate cell cultures and animal models, so it seemed an appropriate agent to evaluate in tissue slice cultures. Although we can only confidently maintain prostate tissue slice cultures for 24 hours at this time, this is sufficient to potentially see effects of 1,25D. Tissue slices have been treated +/- 1,25D and are ready for histologic and immunohistochemical evaluation.

Our results in this first year support our contention that development of prostate tissue slice cultures will be feasible.

## **REFERENCES**

None.

## **APPENDICES**

Figure legend and Figures 1-7.

## FIGURE LEGENDS

Figure 1. Tissue slices were cultured in either Complete PFMR-4A medium (panels a, c, e, g) or in Complete MCDB 105 medium (panels b, f, d, h) for 24 (panels a, b, e, f) or 48 (panels c, d, g, h) hrs (panels a, b, c, d 100X, panels e, f, g, h 400X). After culture, tissue slices were fixed, embedded, sectioned and stained with H & E. Tissue structure appeared to be better maintained in Complete PFMR-4A over time compared to Complete MCDB 105.

Figure 2. Immunohistochemical evaluation of cytokeratins 5 and 18 in tissue slices cultured as shown in Figure 1. Slices in panels a, c, e and g were cultured in Complete PFMR-4A, whereas slices in panels b, d, f and h were cultured in Complete MCDB 105. Slices in panels a, b, e, and f were cultured for 24 hr, whereas slices in panels c, d, g and h were cultured for 48 hrs (panels a, b, c, d 100X, panels e, f, g, h 400X). In all cases, keratin 5 was appropriately expressed in basal epithelial cells and keratin 18 in secretory epithelial cells, showing maintenance of function. However, the maintenance of structure was better in Complete PFMR-4A.

Figure 3. Immunohistochemical evaluation of smooth muscle  $\alpha$ -actin in tissue slices cultured as shown in Figure 1. Slices in panels a and e were cultured in Complete MCDB 105 for 24 hrs, or in Complete PFMR-4A for 24 hrs (panels b and d) (panels a and b 100X, and panels e and d 400X). Smooth muscle actin maintained appropriate expression in the stroma in both cases.

Figure 4. Tissue slices from a different specimen from that shown in Figures 1-3 were cultured in Complete PFMR-4A with (panels b and f) or without (panels d and h) 10 nM R1881 for 24 hrs. Panels a, e, c and g show tissue slices that were not cultured but were immediately fixed after slicing. Immunohistochemical staining was done for cytokeratin 18. Although keratin 18 expression was maintained with or without R1881, tissue structure appeared to be better maintained with R1881. (Panels a-d 100X, panels e-h 400X).

Figure 5. Tissue slices cultured as shown in Figure 4 were maintained for 48 hrs in Complete PFMR-4A with (panels b and f) or without (panels d and h) 10 nM R1881 and compared to uncultured slices (panels a, e, c and g) after immunostaining for keratin 18. After 48 hrs, the tissue degeneration seen at 24 hrs in the absence of R1881 was even more pronounced, although keratin 18 expression was maintained. (Panels a-d 100X, panels e-h 400X).

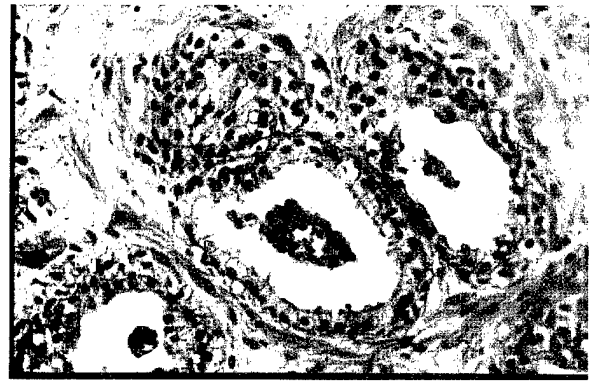
Figure 6. Tissue slices cultured as shown in Figure 4 were maintained for 24 (panels a, b, e, f) or 48 (panels c, g, d, h) hrs in Complete PFMR-4A with (panels a, e, c, g) or without (panels b, f, d, h) 10 nM R1881. Immunostaining for PSA revealed continued expression of PSA in all cases, but tissue degeneration was more pronounced in the absence of R1881 at 48 hrs. (Panels a-d 100X, panels e-h 400X).

Figure 7. Tissue slices obtained from a different specimen than those shown in Figures 1-6 were cultured in Complete PFMR-4A with 10 nM R1881 and with (panels c and d) or without (panels e and f) 50 nM 1,25-dihydroxyvitamin D3 for 24 hrs. Fixed sections were stained with H&E and compared to uncultured slices (panels a and b). No significant effects of vitamin D on tissue structure were noted. (panels a-e 100X, panels b-f 400X).

Fig. 1



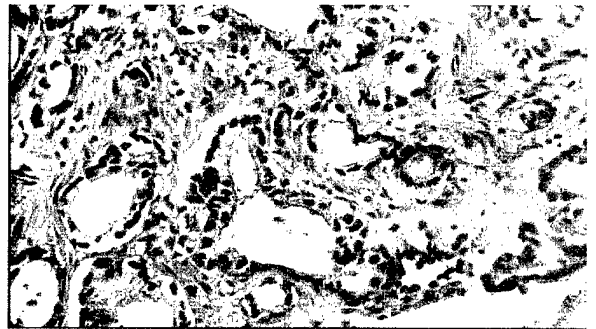
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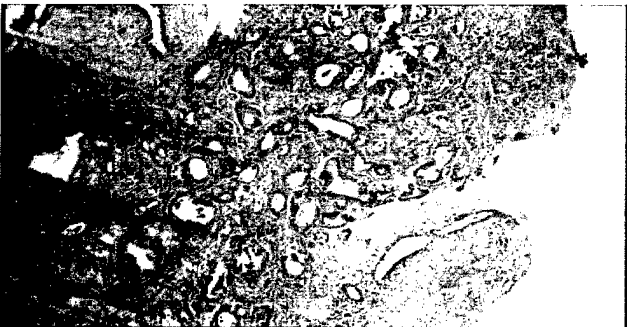
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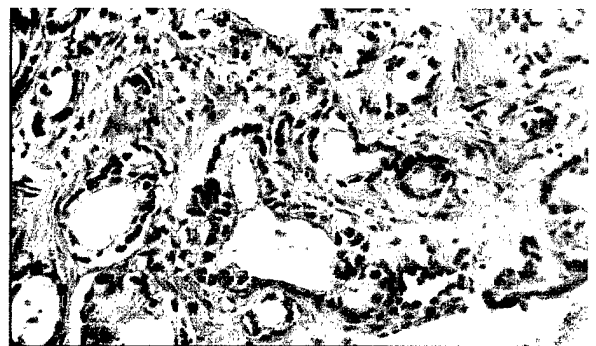
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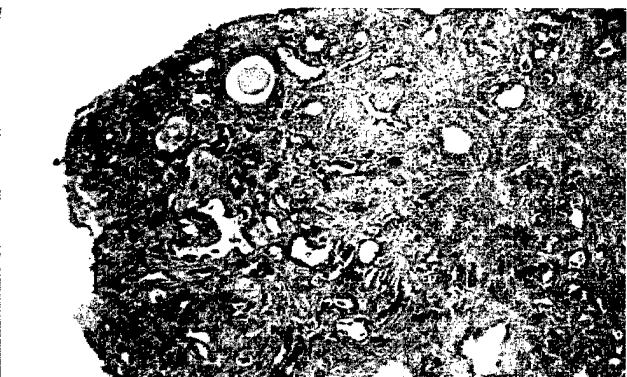
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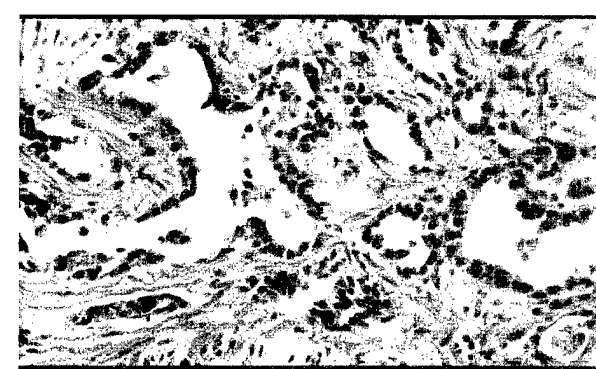
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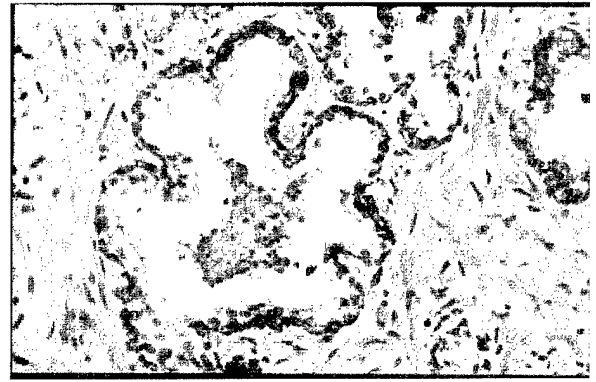


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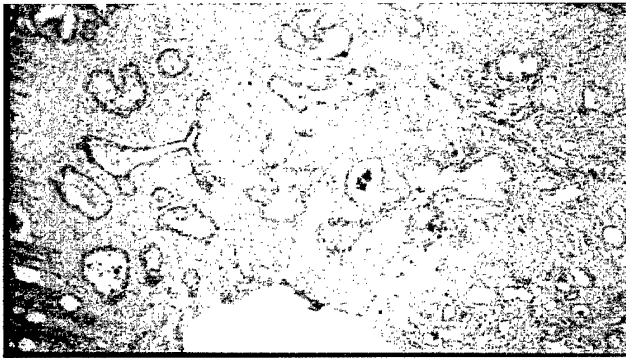
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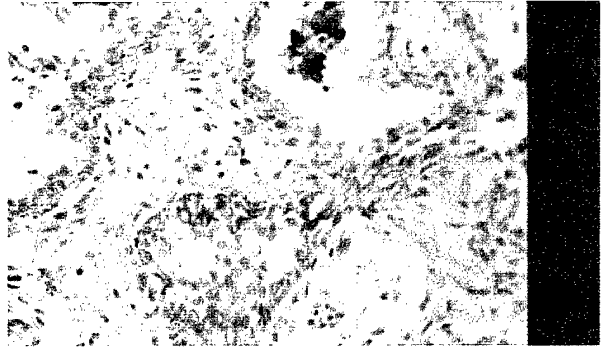
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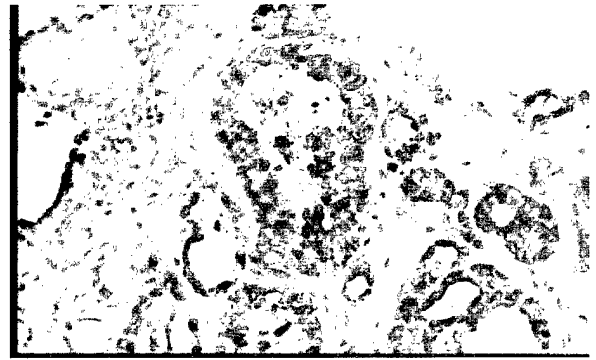
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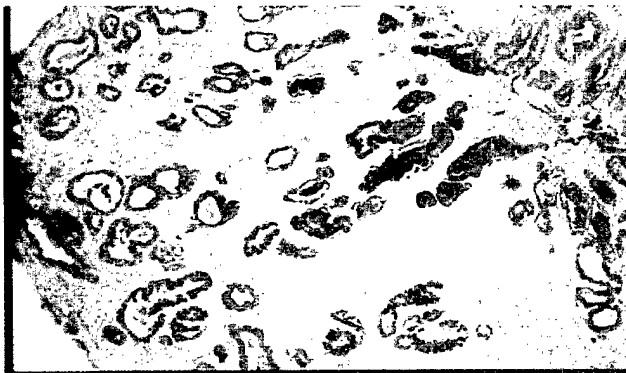
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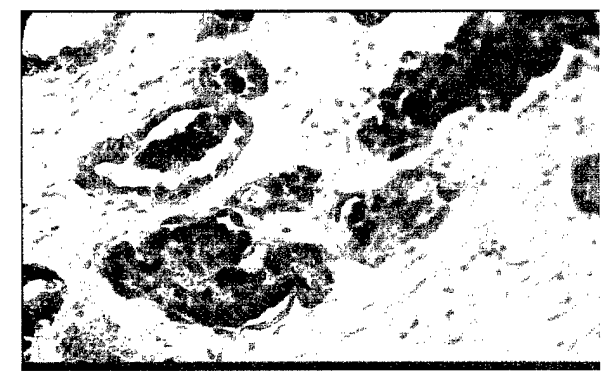
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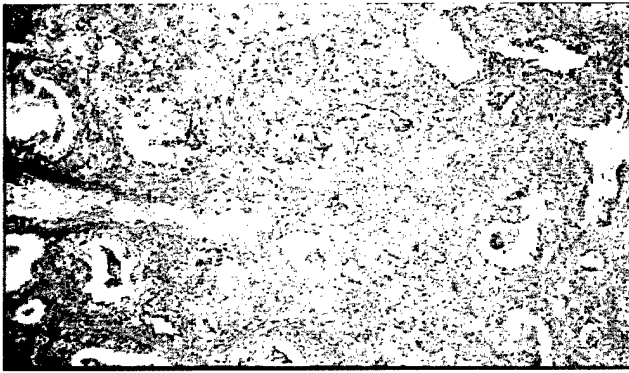


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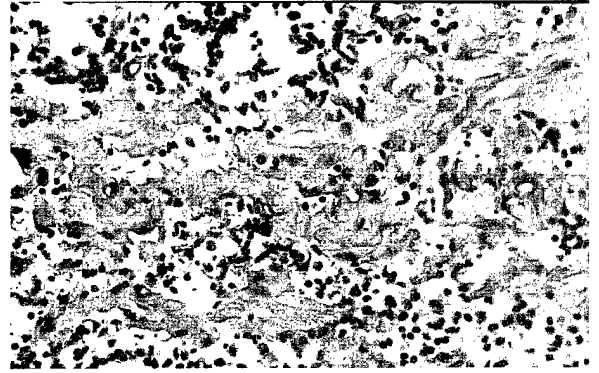


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Fig. 3



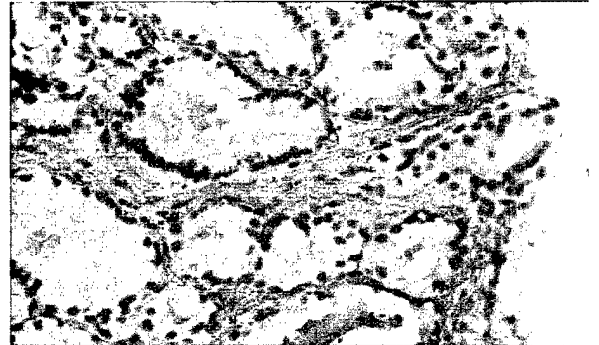
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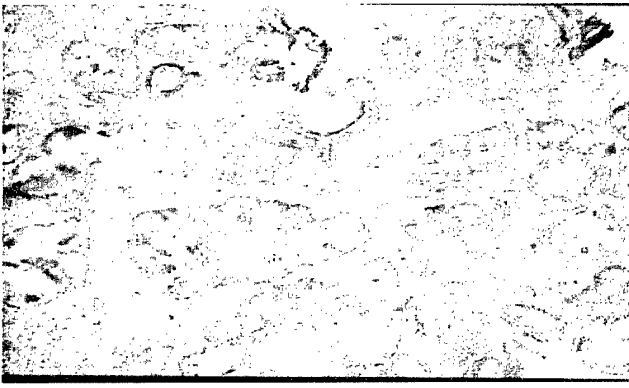


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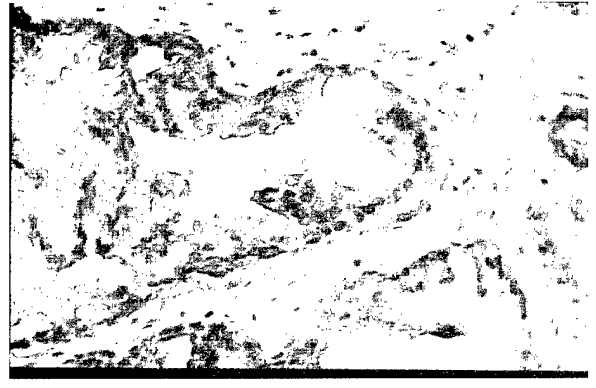


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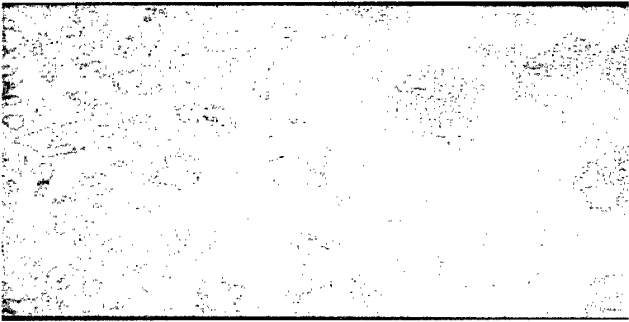
Fig. 4



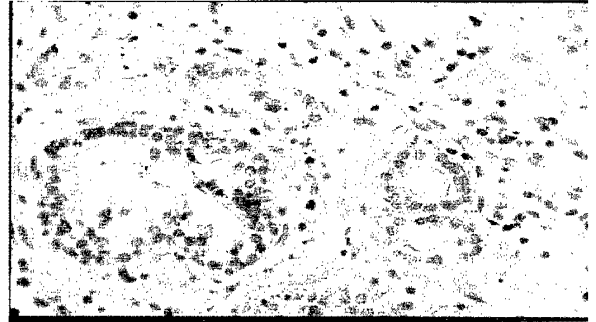
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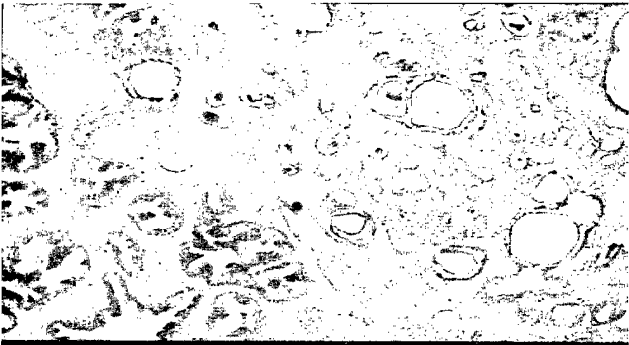
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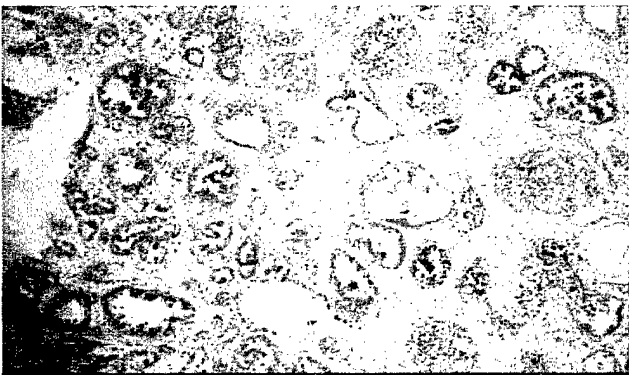
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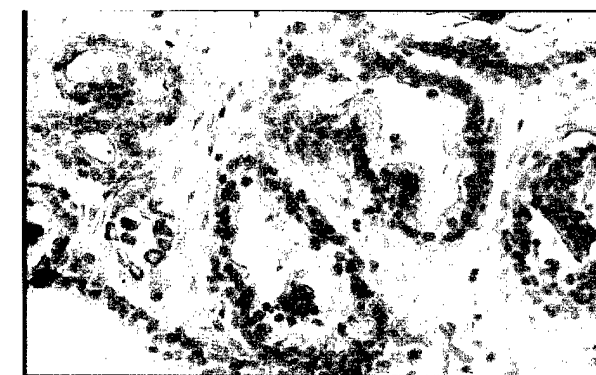
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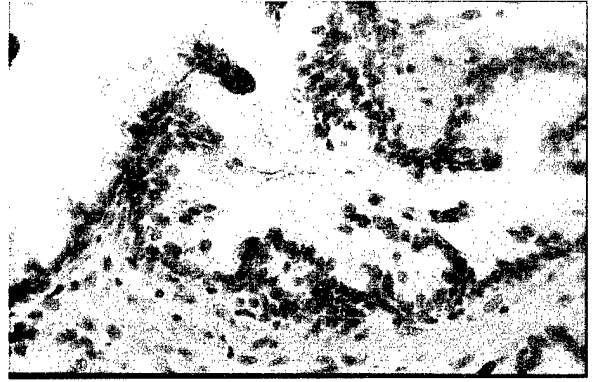


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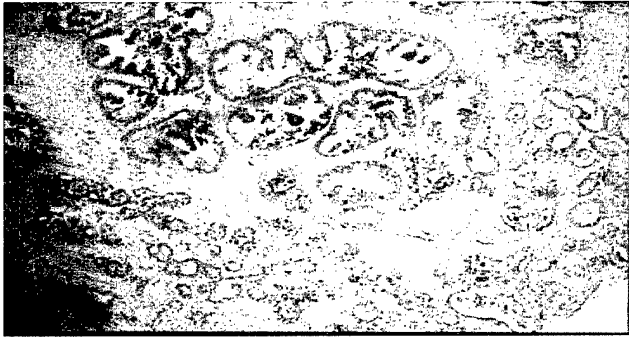
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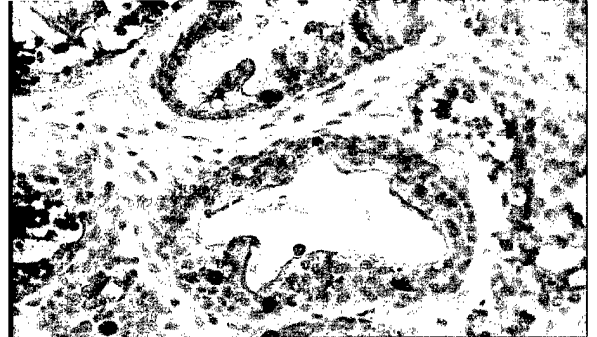
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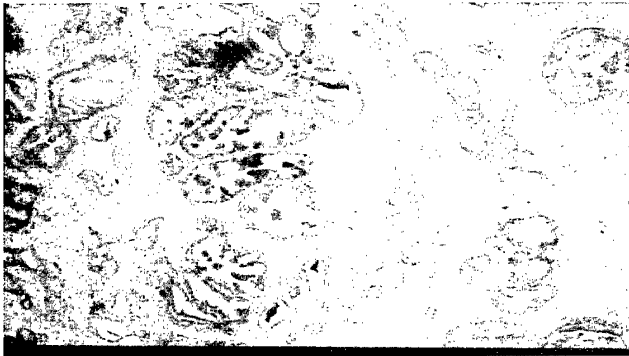
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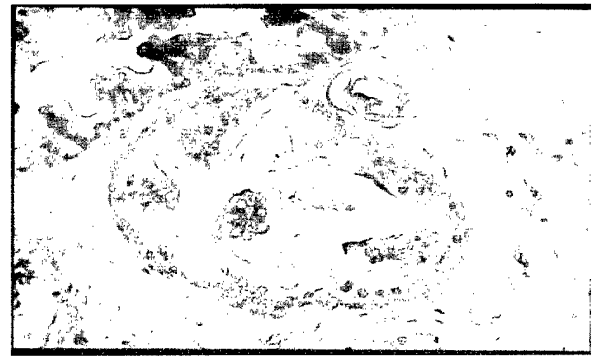
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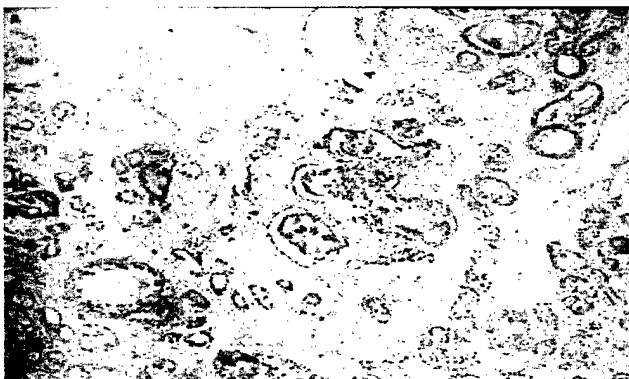
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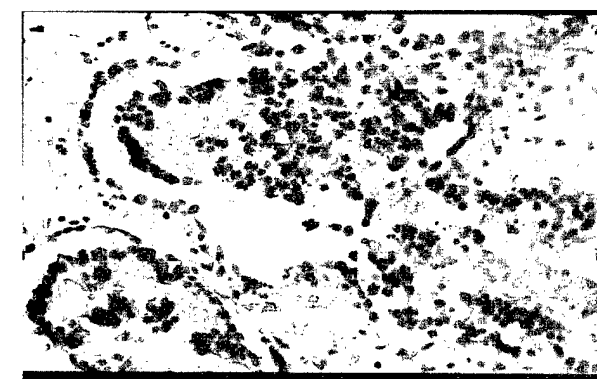
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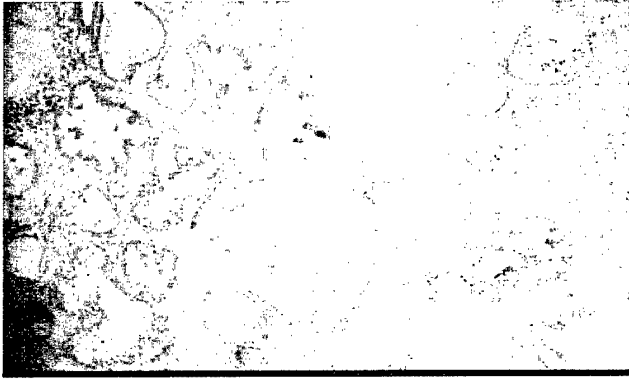


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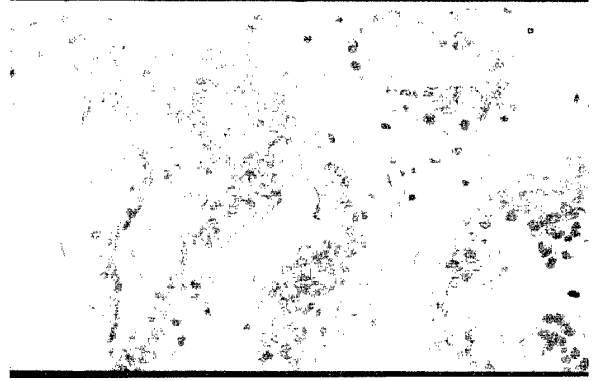


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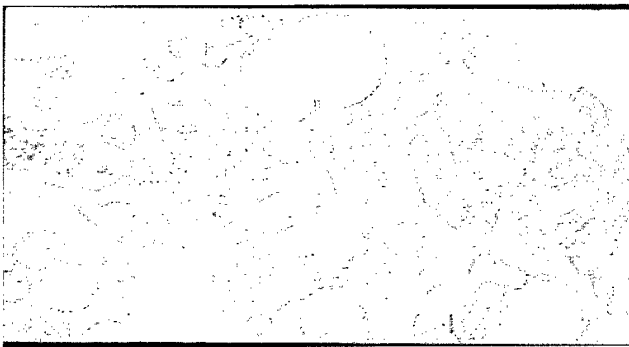
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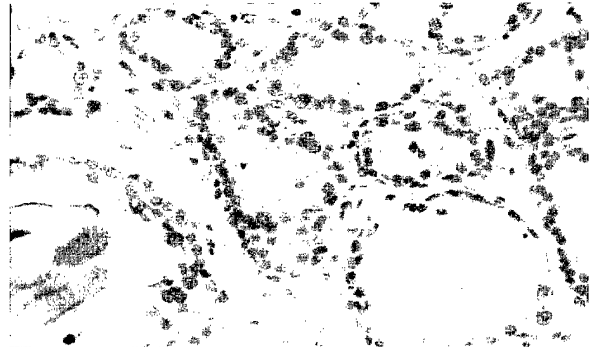
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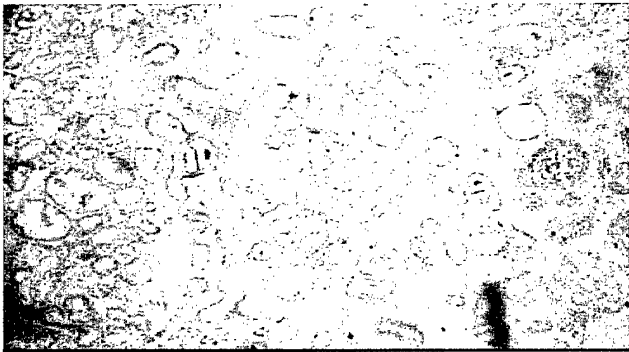
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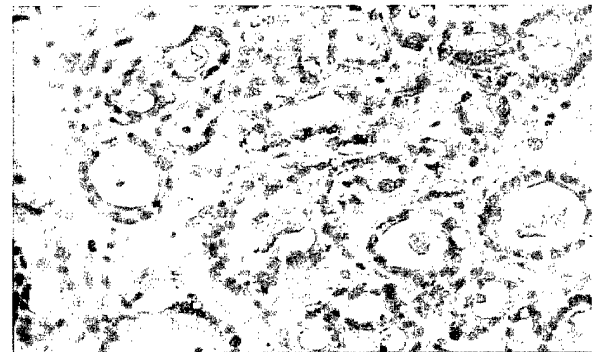
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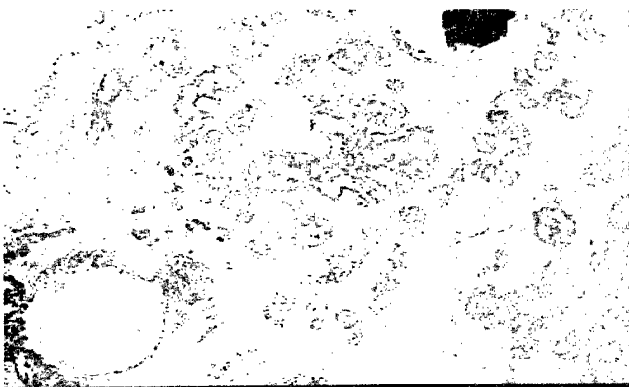
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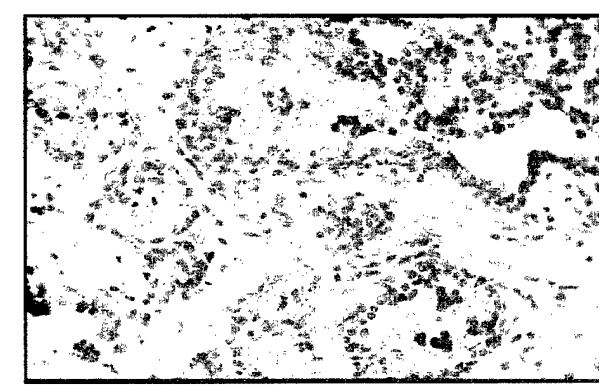
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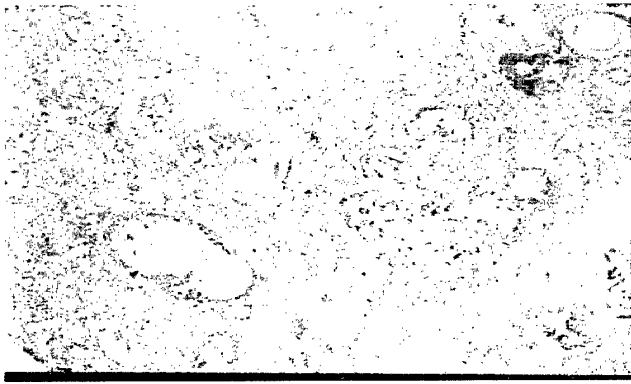


d

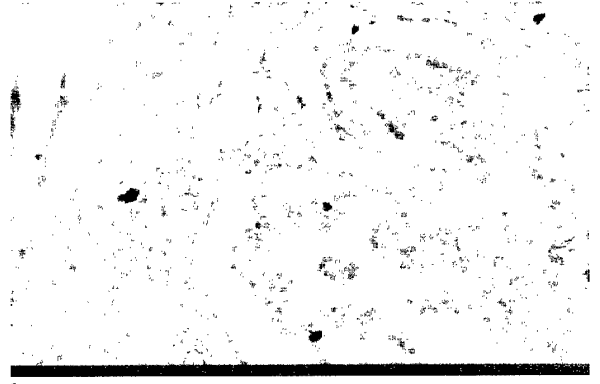


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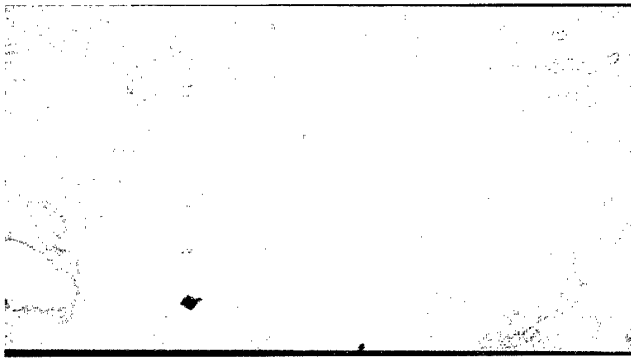
Fig. 7



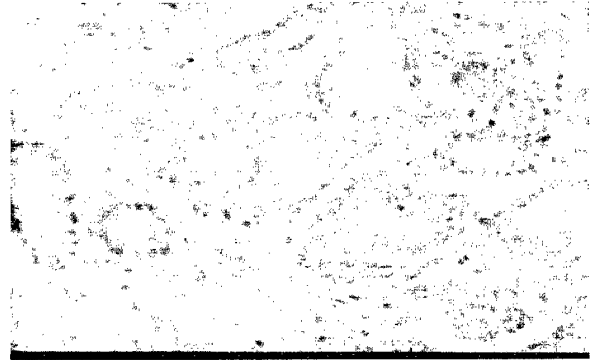
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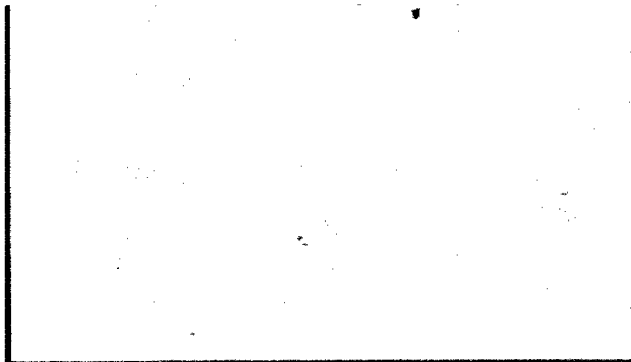
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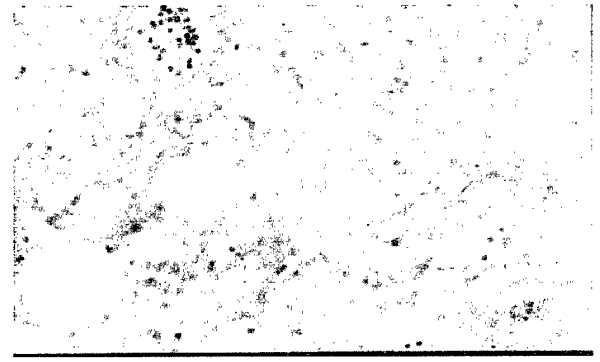
c



d



e



f