THE EFFECTS OF FILTRATION ON STORED HUMAN BONE MARROW

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INTRODUCTION

During the last five years, we have been studying the effects on human beings of a single exposure of total body radiation which has been given for the palliation of metastatic malignancy. To date, forty patients have been so treated with 25 to 200 rad midline absorbed tissue dose (41 to 336 R midline air dose) of total body radiation. Eighteen of these patients were given 150 to 200 rad.

Marked hematologic depression occurred in all eighteen patients who received more than 125 rad total body radiation. The total leucocyte count fell below 2000 WBC/mm^3, 25 to 40 days after irradiation. The mean minimum leucocyte count of previously untreated patients who received 150 rad total body radiation alone was 1264 $\pm$ 1140, and was 1140 $\pm$ 816 when there had been previous therapy. The nadir of the leucocyte counts of patients who received 200 rad was 983 $\pm$ 366 WBC/mm^3.

Since bone marrow depression was the most life threatening radiation effect at the doses used, we began storing bone marrow for autologous infusion prior to irradiation. To date, we have stored marrow from twenty five patients and have infused autologous stored human bone marrow intravenously <u>without filtration</u> into six patients. One patient had hemoglobinuria for twelve hours after infusion at 100 drops per minute. Autopsies were performed in three patients five days, eight days and nine days respectively after reinfusion. No evidence of pulmonary emboli, pulmonary

infarction or other disease as a result of marrow infusion was found on microscopic examination.

METHODS

The bone marrow has been stored by a modification of the method previously described by Kurnick (1). Approximately 100 ml. of material is aspirated from each posterior iliac crest. The marrow suspension is diluted with an equal amount of 30% glycerol -Osgood media. This mixture, in quantities of 10 ml. per culture tube is cooled at 1° Centigrade per minute to -40° Centigrade. The tubes are then placed in polyethylene bags and stored at -80° Centigrade.

On the day of infusion, the frozen marrow-glycerol-Osgood media is thawed at 4° Centigrade. Two parts of it are diluted with one part 33 1/3% dextrose in water and placed in a Fenwal plastic transfer pack. It was this mixture which was infused intravenously without filtration through a 17 gauge siliconized needle.

There is disagreement as to the need for filtration. Kurnick does not think it is necessary prior to infusion. Thomas, Ferrebee and Pillow (2,3,4) recommend filtration to minimize the incidence of pulmonary embolization. Though we have not encountered embolization, we have developed a simple method of filtration for use in man.

The complete infusion filtration system consists of a Fenwal transfer pack, plastic tubing, filter assembly, and Sigmamotor pump (Figure 1). The pump has a variable speed control, but for the experiments to be reported, has been run at 55-60 drops per minute. Within the filter assembly (Figure 2), there are successive number #50, #100 and #200 mesh filters corresponding to openings of 297, 149, and 74 microns. The filter assembly is constructed of stainless steel with Teflon washers so that it can be disassembled for cleaning. It can then be reassembled, sterilized and made pyrogen free.

When the pump was used without a filter assembly, the plastic tubing would separate from the 17 gauge needle because of the large pieces of fibrin and marrow which would occlude the needle. With the complete system, including the filter assembly, a 25 gauge needle has been used without encountering any obstruction at the needle hub.

We have investigated the total cell count of a volume of marrow suspension and the viability of stored marrow cells before and after filtration through the infusion filtration system described. Cell counts are reported as cells per ml. (1). Viability studies, as determined by dye exclusion, utilized trypan blue (5). By a modification of the method of Moorhead (6), the marrow from several patients has been cultured under phytohemagglutin stimulation for 6 days. 1.0 to 1.2 x 10^6 cells per ml. are implanted in a culture media of 70% TC 199 and 30% pooled human

serum (7). On the third day, the marrow is replanted, and additional phytohemagglutin is added. It is thought that the cell which differentiates and undergoes mitosis under these in vitro conditions is a hematopoietic cell.

RESULTS

We have studied bone marrow from fourteen patients. The marrow has been stored from five to twenty six months prior to study. Though the storage temperature has usually been -80° Centigrade, at times, due to transient equipment failure, the temperature has risen to as high as -40° Centigrade for as long as twenty four hours.

Cell counts have been performed on nineteen specimens of bone marrow from fourteen patients before and after filtration (Table I). The mean of the cell counts prior to filtration was 3.8 ± 2.0 cells x 10^6/ml. and 3.0 ± 1.6 x 10^6/ml. after filtration. When these means are compared by an analysis of variance, there is no significant difference in the two groups at the 5% level. The sensitivity of the cells to injury by filtration was not influenced by the duration of storage at low temperatures.

Trypan blue exclusion studies were performed on seventeen specimens of bone marrow obtained from twelve patients (Table II). The mean of the % viability before filtration was $55.3 \pm 8.9\%$ and $55.5 \pm 8.1\%$ after filtration. There was no significant

difference between these two groups. Percent viability as determined by this dye exclusion technique was not influenced by the length of storage up to twenty six months nor by filtration through the system described.

Attempts to culture in vitro seven aliquots of bone marrow from six patients have yielded metaphases in two studies. In one additional study, "large mononuclear cells" with prominent nucleoli and vacuolated cytoplasm were observed. No metaphases were seen. No growth was present in the other four cultures. Figure 3 is an example of a metaphase seen on slides from a culture of bone marrow which had been stored for 5.5 months. A metaphase from the same aliquot of bone marrow after filtration is seen in Figure 4. We have been able to culture marrow which has been stored for 13.5 months. Metaphases have been photographed from slides made from such cultures before filtration (Figure 5), and after filtration (Figures 6 and 7). A large number of metaphases has not been observed, but the presence of some is evidence of a degree of viability of the stored marrow. The number of metaphases did not decrease after filtration.

If the bone marrow has not been filtered, macroscopic clumps of material, as large as 3 mm. in length, are seen passing through the tubing. On microscopic preparations from unfiltered marrow, clumping is visualized (Figures 8 and 9). Following filtration, no macroscopic particles are seen and individual separated cells

are observed on microscopic examination (Figure 10). No clumping is noted on this slide which is from the same marrow study as the two with sheets of cells.

DISCUSSION

One of the first tenets in the handling and preparation of human tissue is to minimize external manipulation whenever possible. This thought led us to the use of unfiltered marrow in the past. However, there was little question that filtered marrow would have advantages over unfiltered marrow if the ability to repopulate the marrow space was not altered by filtration. We have demonstrated that the infusion filtration system used in these experiments is easier to use than the system without the filter assembly. With the filters, a needle as small as a 25 gauge can be used for the intravenous infusion.

Since there has been no significant alteration in the cell count nor in the viability of the cells when studied in vitro, there does not appear to be a deleterious effect from filtration. Only two in vitro tests of viability were used, namely the exclusion of trypan blue dye and the capacity to grow cells in tissue culture with subsequent demonstration of mitoses. Further investigation of the cellular viability with tritium labelling of cells and acridine orange studies is advisable.

The ultimate answer as to whether filtration alters the ability of stored autologous human marrow to repopulate the marrow can come only from an adequately controlled investigation in man.

This study supports the view that filtered autologous marrow should be effective in the management of myelosuppression due to radiation therapy, chemotherapy or accidental radiation exposure in industry, in warfare, or in space.

SUMMARY

An easy-to-use infusion filtration system for autologous stored human bone marrow has been developed. There has been no difference in the means of the cell counts of marrow before or after filtration in nineteen experiments. In vivo viability studies were not changed by filtration in seventeen studies. Viability was unchanged by six to twenty six months storage at -80° Centigrade. After tissue culture for six days under phytohemagglutin stimulation, metaphases were observed in specimens obtained before and after filtration.

This study supports the concept that filtered autologous human bone marrow would be effective in controlling the severe hematologic depression associated with chemotherapy or radiation injury.

Table I

Case Number	Storage Time (months)	Cell Count (x 10^6/ml) Before Filtration	After Filtration
068	5.5	5.6	4.1
065	5.5	1.6	0.8
067	6	4.2	3.3
065	7	1.4	0.6
066	10	5.3	4.4
064	11.5	4.8	4.1
064	12	3.6	3.1
063	13.5	9.8	8.2
056	17	3.5	2.9
050	21	6.2	2.6
050	21.5	2.2	2.4
052	22	3.2	3.5
052	22.5	3.2	3.5
047	23	4.4	3.2
049	24	4.3	3.6
048	25	1.8	1.4
x1	26	3.0	2.5
x2	26	3.0	2.2
047	26	1.3	1.2
Mean ± S.D.		3.8 ± 2.0	3.0 ± 1.6

Table II

Case Number	Storage Time (months)	% Viable Cells Before Filtration	% Viable Cells After Filtration
068	5.5	69	70
065	5.5	--	--
067	6	58	46
065	7	--	--
066	10	51	56
064	11.5	53	54
064	12	64	60
063	13.5	63	57
056	17	63	56
050	21	38	36
050	21.5	49	46
052	22	52	69
052	22.5	47	55
047	23	44	58
049	24	69	52
048	25	63	64
x1	26	60	52
x2	26	52	60
047	26	45	53
Mean ± S.D.		55.3 ± 8.9	55.5 ± 8.1

Figure 1

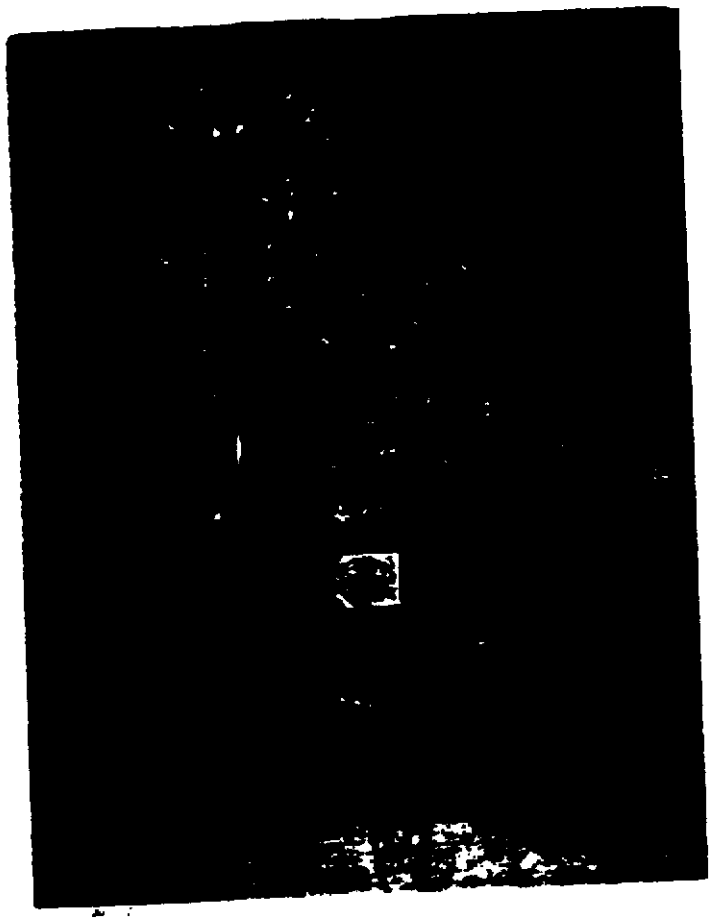


Figure 2

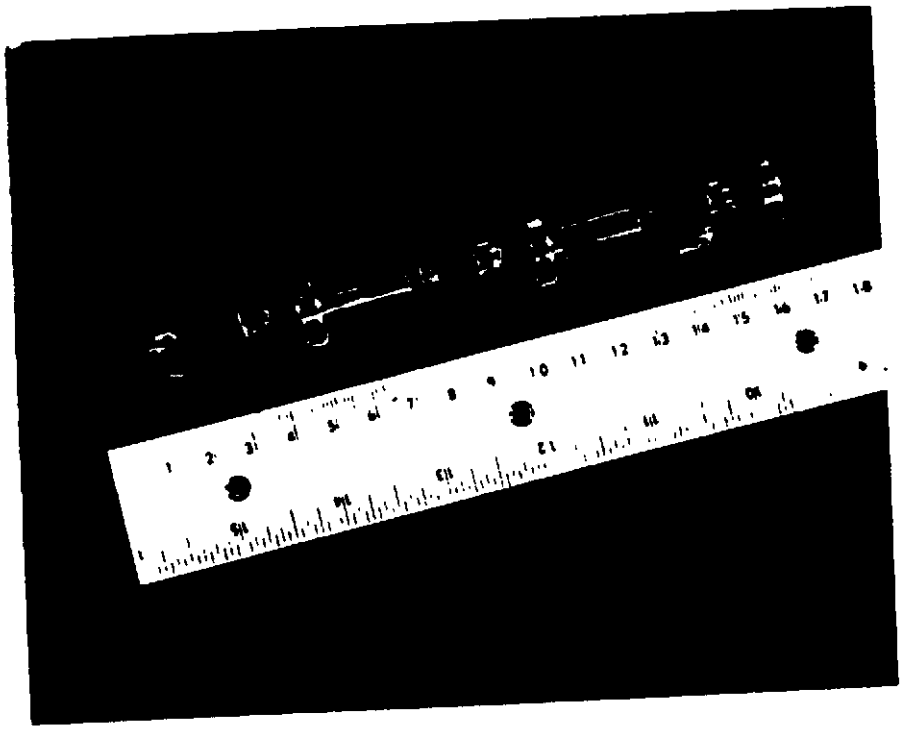


Figure 3

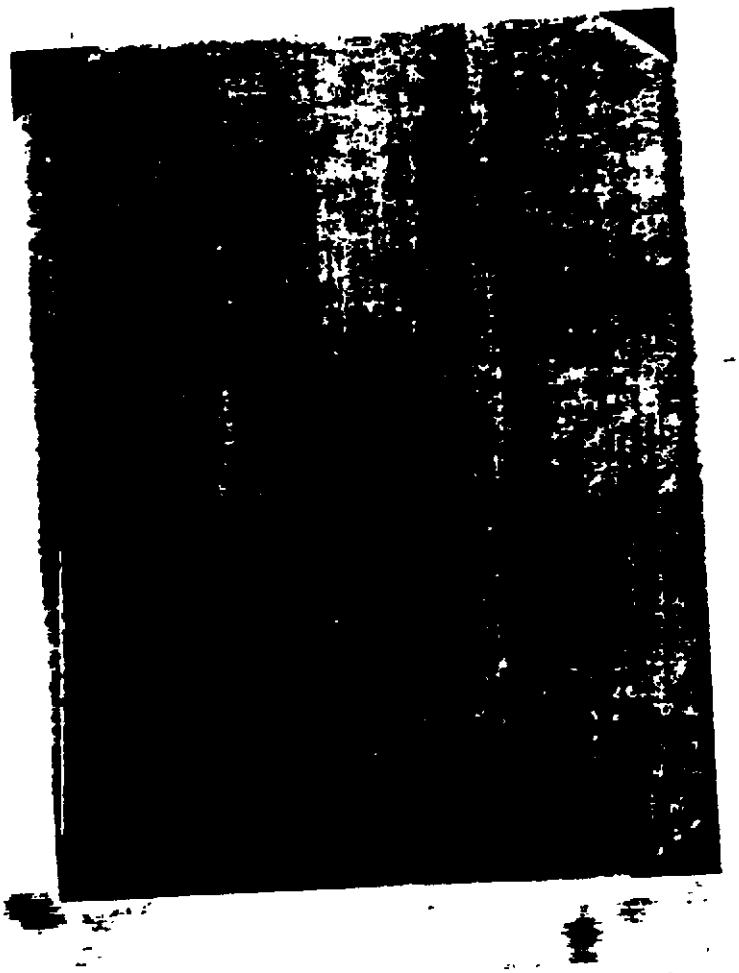
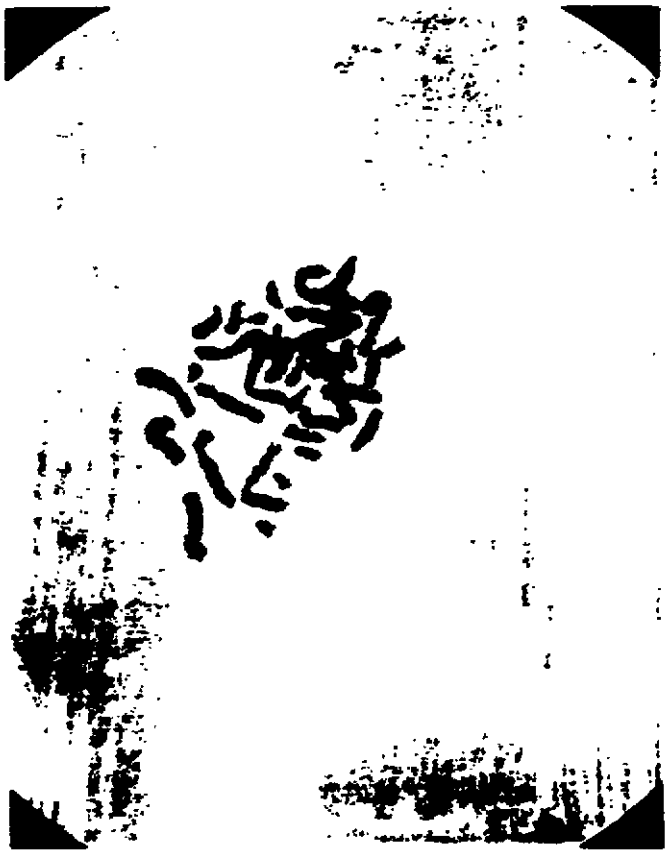
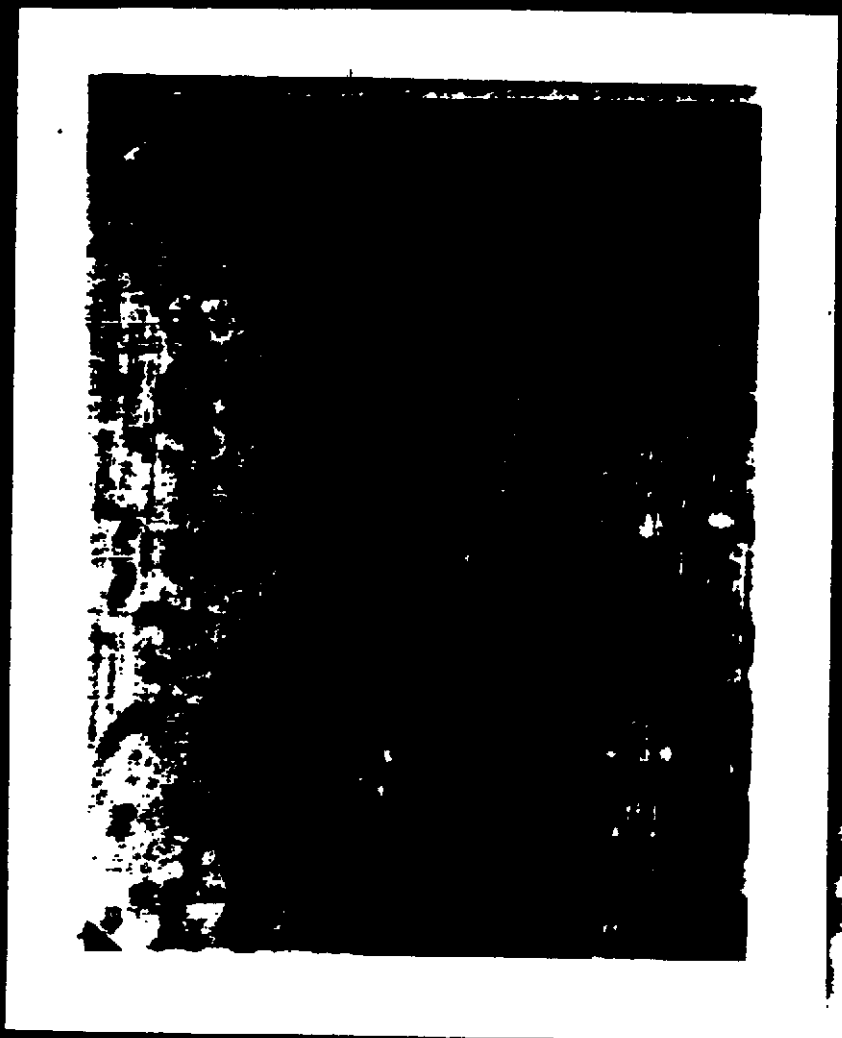


Figure 3





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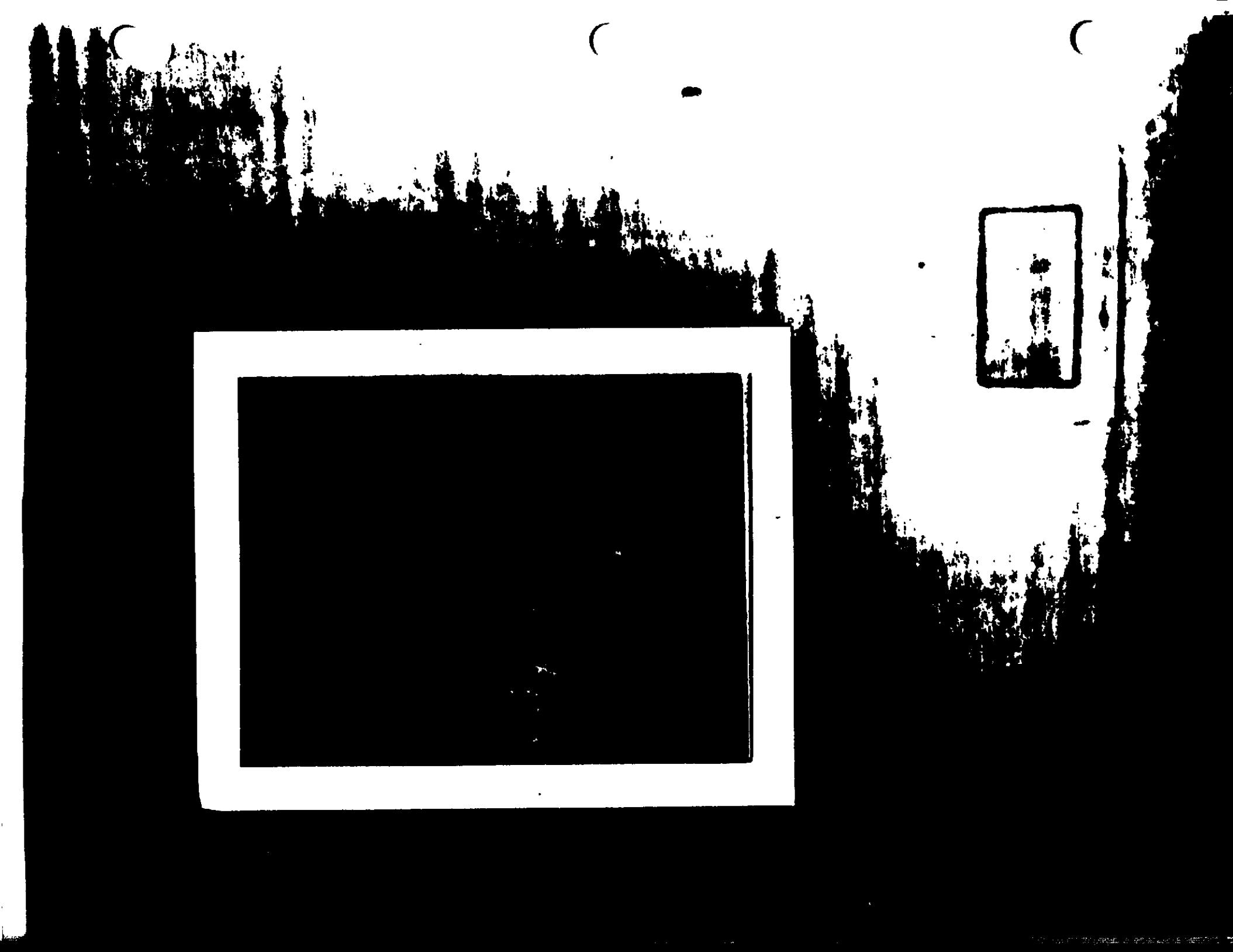

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