EFFECTS OF PURIFIED AND NON-PURIFIED PAPER PLANT EFFLUENTS ON FERTILIZATION AND DEVELOPMENT OF SEA URCHIN *Paracentrotus lividus* Lam., EGGS.

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Abstract. The gametes and embryos of *Paracentrotus lividus* were exposed to various dilutions of purified and non-purified effluents of a paper plant with sea water. As the salinity of effluents resemble much the tap water, tap water was also diluted with sea water and used as test solution to observe pathologic features which may be due to decrease in ionic strength. Success in attaining pluteus was higher in non-purified effluent after 48 hours at lowest concentrations (15% and 20%) than in purified effluent. In contrast, formation of fertilization membranes and further development at higher concentrations was peculiar to purified effluent. Apart from the effluents, tap water severely suppressed the development and even at the lowest concentration (15% tap water + 85% filtered sea water) caused cytolysis after 48 hours. Only 2% of the fertilized eggs have succeeded gastrulation. The toxicity of purified and non-purified effluents were resembled, yielding quite better conditions in the purified. Following fertilization of the eggs further development was fully stopped and pathologic features were permanent at all eggs at the 30% non-purified effluent concentration. In general, developmental defects were increased with decreasing salinity at all test solutions. However, the pH of the test solutions did not vary significantly with decreasing salinity.

Introduction

Sea urchins long have stimulated the curiosity of many researchers especially of those intended to study reproduction and development. Embryogenesis in the sea urchin has been intensively studied for more than a century. Few studies aimed to study solely the embryology of echinoids from fertilization through pluteus in the past several decades [1]. Besides these, enormous number of experiments were conducted on the biological effects of various agents on germ cells and embryos of sea urchins [2 and references cited there in]. The use of sea urchin eggs and embryos as an indicator in marine pollution bioassay was first proposed by Kobayashi in 1971 [3]. Studies concerning the effect of effluents from paper mills are very scarce and to the best of authors knowledge, the effect of tannic acid derived from timber-reservoirs or lumber industry was only pronounced earlier by Kobayashi [4]. This study aims to fulfill this gap via studying the effect of purified and non-purified effluents of a paper mill on various developmental stages of *Paracentrotus lividus* Lam., through pluteus.

Materials and Methods

a) Organisms

Adult sea urchins were collected from the Institute harbour endemic of the region. Specimens were than kept in tanks over night at a thermostatically controlled room (18-20°C) for acclimitization. Rippen eggs and sperms from mature individuals were obtained by the current KCl-method [5]. Female specimens remained perched on glass beakers filled with filtered seawater to the top to release eggs for 15 minutes. Sperms from testes were collected into empty glass beakers to preserve dry. Mature eggs from several females

(usually 3-4) were then pooled and rinsed twice with filtered sea water. Following this, first the eggs were inseminated to one of the duplicate test solutions 15 minutes prior to the addition of sperms to the other solution. Fertilization was initiated after exposure of eggs (30 minutes) and sperms (15 minutes) to solutions by pouring sperms on eggs. The percentage of the eggs with raised fertilization was checked shortly after in first sampling within 15 minutes. Second sampling is done after 48 hours at the stage of pluteus. All samples were preserved in 10% buffered formalin. In addition salinity and pH of each test solution were measured. separately.

b) Test solutions

Purified and non-purified effluents were collected from a nearby Paper Plant (SEKA-Taşucu) (Figure 1) as the test solutions for comparison. Main products and consumption of the plant are listed below.

Products	Consumption
kraft paper	sulfate cellulose
kraft liner	resin glue
sulfate cellulose	alum
kraft paper cellulose	sodium hydroxide
kraft liner cellulose	sulfuric acid
white liquor	hydrochloric acid
terebenthene	disodium phosphate
tall oil	sodium sulfate
	sodium sulfite
	sodium carbonate
	sodium chloride

Concentration of substances measured in the purified and non-purified effluents are given in Table 1.

Table 1. Average values of the chemical parameters of monthly 24 hour composite purified and non-purified effluents [6].

Parameters	Non-purified	Purified
COD (mg/l)	1262	393
BOD (mg/l)	375	183
TSS (mg/l)	564	88
pH	7.2	7.4
NO3+NO2 (mg/l)	0.07	0.06
O-PO4 (mg/l)	0.07	0.04
Total phosphorus (mg/l)	0.09	0.05
Total Hg (ppb)	- -	0.005
Co (ppm)	0.048	0.048
Cu (ppm)	0.003	0.003
Pb (ppm)	0.075	0.150
Ni (ppm)	0.069	0.069
Zn (ppm)	0.013	0.020
Fe (ppm)	0.84	0.74
Mn (ppm)	0.242	0.174
Cr (ppm)	0.023	0.018

Effluent samples were 24 hour composites at two hour intervals. These were than filtered with GF/B Glass Microfibre filters to remove particles. Natural sea water was collected from the sample collection site in the harbour and used as the control and the diluent. Tap water was aerated for 24 hours in order to remove excess chlorine and kept over night in an open glass jar to stabilize the oxygen level. Test solutions were obtained by dilution of tap water, purified and non-purified effluents with filtered natural sea water. Initially performed range determination experiments have shown that the effluent concentrations below 15% (85% sea water + 15% effluent) have had no harm on development of eggs. For the bioassay, effluent concentrations in the test solutions ranged between 15 to 50%. Experiments were conducted separately for each test solution and for control with three replicates.

Results and discussion

The response to tap water, purified and to non-purified effluent of *Paracentrotus lividus* is summarized in Tables 2, 3 and 4. The duration of the bioassay covers 48 hours and the percentage of success given is the mean of three replicates. Microscope observations on developmental stages have aimed formation of fertilization membrane, polyspermic cleavage, morula, blastula, gastrula and pluteus.

Purified effluent

It is observed that the purified effluent is the least effective in comparison to non-purified effluent and to tap water. Serial dilutions up to 40% (Table 2) have yielded large permanent fertilization membranes at 50% of the eggs within 15 minutes (Figure 2a) similar in shape to those of control (Figure 2b). Over this level the success was below 10% and fertilization membranes were hardly seen under microscope and disappeared almost at all eggs at 50% dilution having a salinity of 17.5 ppt. After 48 hours, 56% of the fertilized eggs reached pluteus and the rest were at gastrula in the 15% effluent concentration. However, 98% of the eggs have reached pluteus in controls at the end of the same period (Figure 2c). No more pluteus was attained at dilutions 25% and over. It was observed that development of eggs are strongly regulated by changes in salinity. Few eggs have succeeded blastulation and gastrulation at 30% dilution. Over this dilution all eggs were irregularly divided, in the multi-cell state due to polyspermy (Figure 2d), and almost all were severely deformed at higher concentrations at a salinity of 22.65 ppt and below. In other words, eggs were highly susceptible to desalinification.

Table 2. Effects of purified effluent on fertilization and development of *Paracentrotus lividus*.

Stage \ Conc.	15 min. after	48 hours after insemination				pH	Sali-nity
	Fertilized eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.98	34.50
15%	98			44	56	7.98	29.42
20%	98	4	18	63	12	7.91	27.73
25%	96	3	21	57		7.96	26.03
30%	90		6	8		7.92	24.34
35%	73					7.96	22.65
40%	51					8.01	20.95
45%	10					7.90	19.26
50%	1					8.03	17.57

Non-purified effluent

Retardation and deformations at various stages of development were more conspicuous in comparison to purified effluent. ~50% of the eggs were able to carry on fertilization in the first 15 minutes at 30% effluent concentration (Figure 3a). Further development to pluteus was prohibited at 25% effluent concentration and majority stayed at gastrulae (Figure 3b). Formation of the fertilization membrane has influenced much at 35% dilution level and stopped totally over this level (Table 3). Following fertilization, critical level for further development was up to 25% effluent concentration around 26 ppt salinity and below this level started deformations and polyspermy (Figure 3c).

Table 3. Effects of non-purified effluent on fertilization and development of *Paracentrotus lividus*

Stage \ Conc.	15 min. after	48 hours after insemination				pH	Sali-nity
	Fertilized eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.96	34.50
15%	100		1	5	94	8.00	29.42
20%	99		5	26	69	8.06	27.73
25%	95	9	21	70		8.18	26.04
30%	52					8.26	24.34
35%	18					8.26	22.65
40%	1					8.27	20.96
45%	1					8.25	19.27
50%	0					8.30	17.57

Very small difference in between effects of non-purified and purified effluents was observed (Tables 2 and 3). As the salinity and pH values of both effluents were almost the same, this effect may be due to another factor or factors. Below a certain level of salinity, fertilization and development of eggs were inhibited and with decreasing salinity the development is totally stopped. Such retardation was assumed to be salinity dependent. Oshida's [7] experiments on purple sea urchin *Strongylocentrotus purpuratus* have shown that fertilization and normal 48-hour development were reduced at salinity levels lower than 28 ppt. The present results are almost consistent with information given by Oshida et al., in terms of the lower limit of salinity effective on development.

Present study clearly showed that none of the fertilized eggs have attained pluteus below 27.7 ppt after 48 hours. Kobayashi (1971) stressing the importance of salinity measurements in these types of bioassays, has observed anomalies during fertilization at densities over and below the range 1.020 to 1.028 [8]. Pagano et al., [9] had also reported highly pathological larvae, reared at 75% salinity while working with cadmium. Results of the present work and others clearly depict that salinity display a significant role on fertilization and development of sea urchin eggs.

Tap Water

Most severe pathologic cases were evident with the tap water when compared with the purified and non-purified effluents. 15 minutes later 100% success was achieved in the formation of clearly visible fertilization membranes (Figure 4a) in controls and in the lowest concentration of tap water (Table 4). With increasing tap water concentrations, very thin, barely seen fertilization membranes became apparent (Figure 4b) and the rate of fertilization decreased and almost failed at the 35% concentration. After 48 hours the picture was extremely terrible and even at the lowest concentration of tap water (15%) cytolysis and polyspermy (Figure 4c) were recorded in almost all eggs while 98% of the eggs have reached pluteus (Figure 4d) in controls during this time. Such a pathologic case was not expected and is challenging. It may be possible that some other factors would effect the fertilization and development of eggs besides salinity. Among these factors, chlorine and trace elements were regarded to be effective. Experiments conducted on sand dollar *Dendraster excentricus* exposed to both chlorinated and unchlorinated West Point, Seattle, Washington effluent prior to fertilization by Stober et al., (1977, cited in [7]) have showed that fertilization was reduced by 50 percent in 4.4% chlorinated effluents. In

addition. Kobayashi (1977) has stressed that trace amounts of heavy metals were effective on developing sea urchin eggs [10].

Table 4. Effects of tap water on fertilization and development of *Paracentrotus lividus*

	15 min. after	48 hours after insemination				pH	Sali-nity
Stage \ Conc.	Fertilized eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.86	34.50
15%	100			2		8.02	29.37
20%	92			1		7.99	27.66
25%	77					8.01	25.95
30%	28					8.01	24.23
35%	1					8.07	22.52
40%	0					8.07	20.81
45%	0					8.07	19.10
50%	0					8.04	17.39

Regarding the effects of pH, it is pronounced earlier that normal fertilization, cleavages and further embryonic development is achieved in the range from 7.8 to 8.6 [4]. As displayed in tables 2, 3 and 4, the pH of the test solutions ranged between 7.86-8.3 in this study. This indicates that any parameter other than pH is controlling the developmental stages. Considering high degree of similarity in salinity and pH levels at all test solutions, crucial chemical analyses of the tap water which strongly

inhibited the development at earlier stages is necessary.

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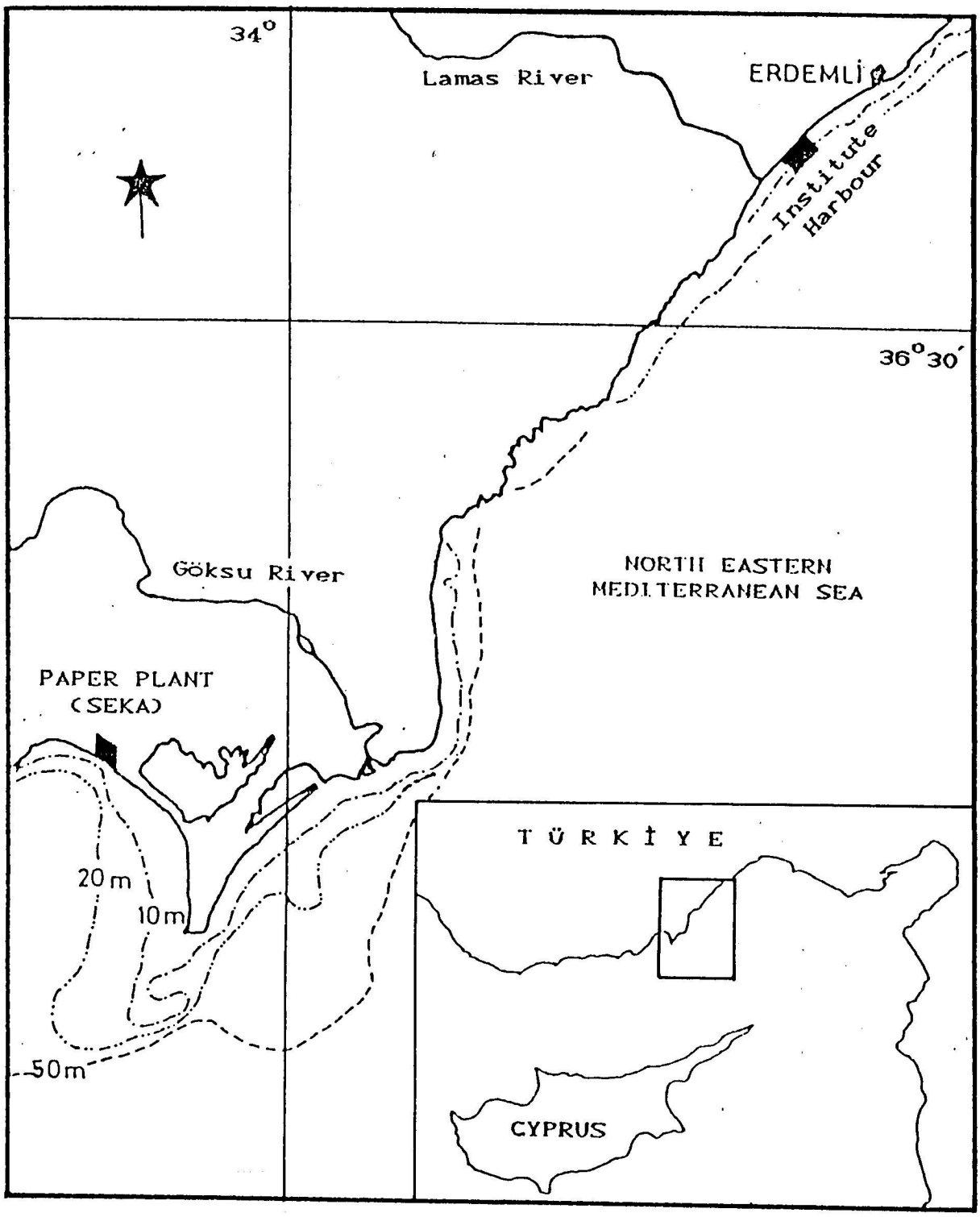
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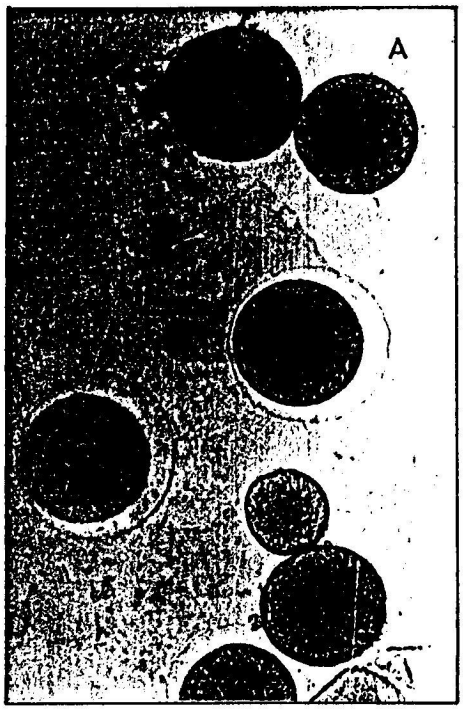
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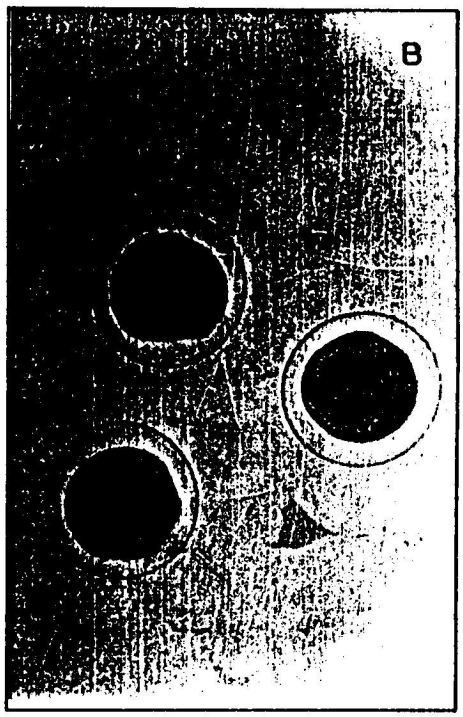
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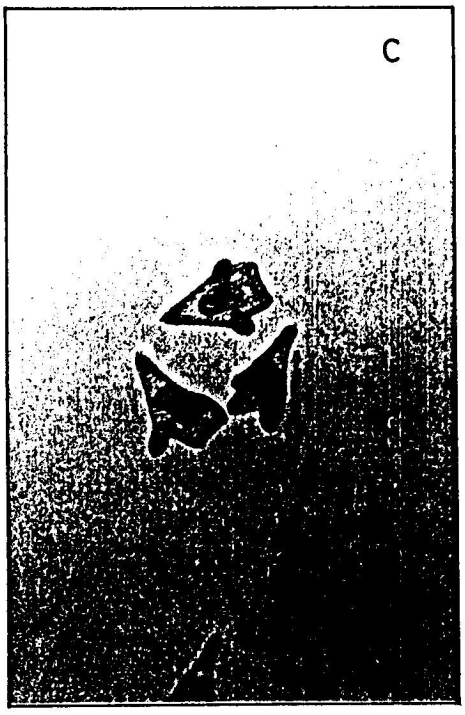
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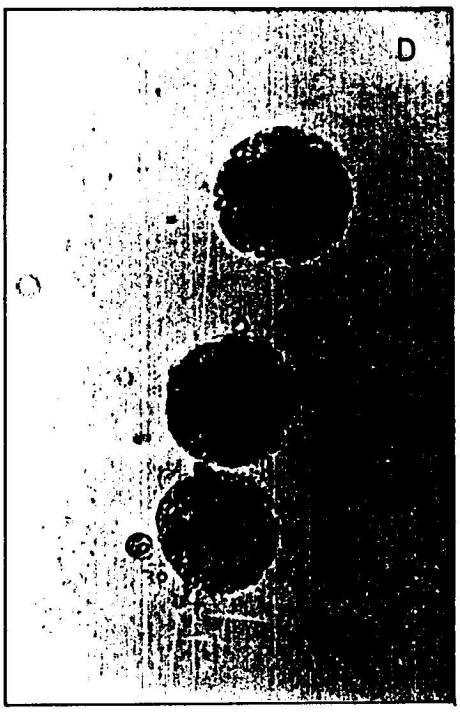
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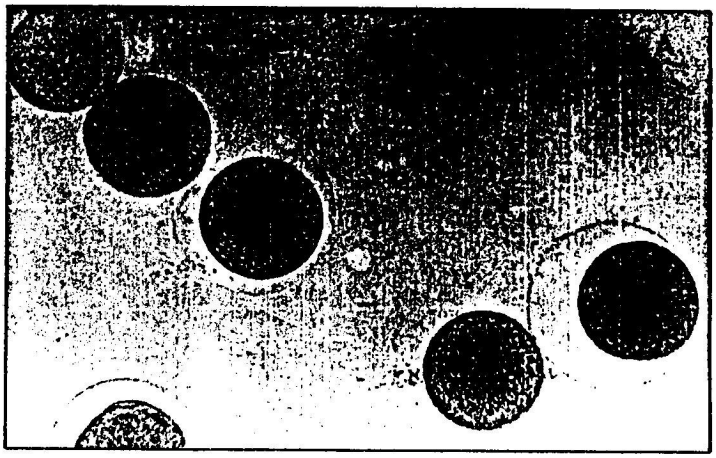

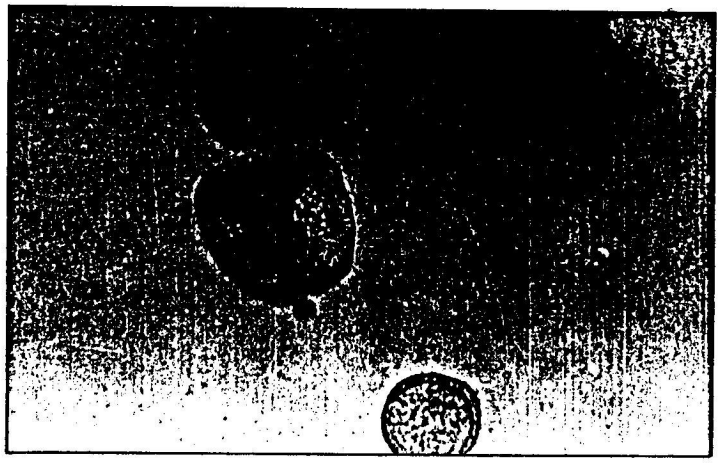

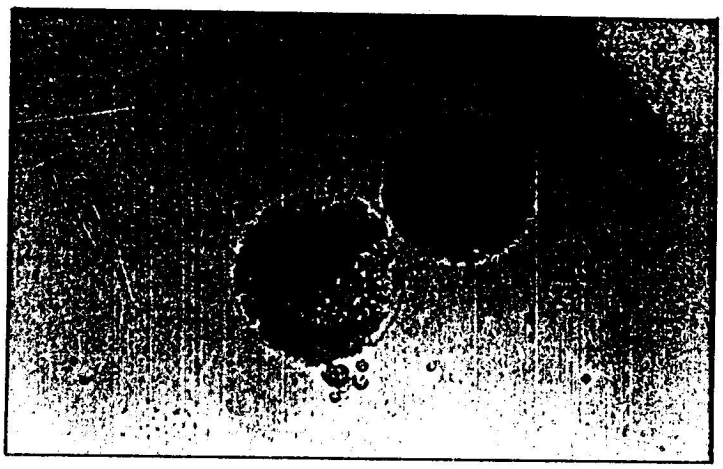

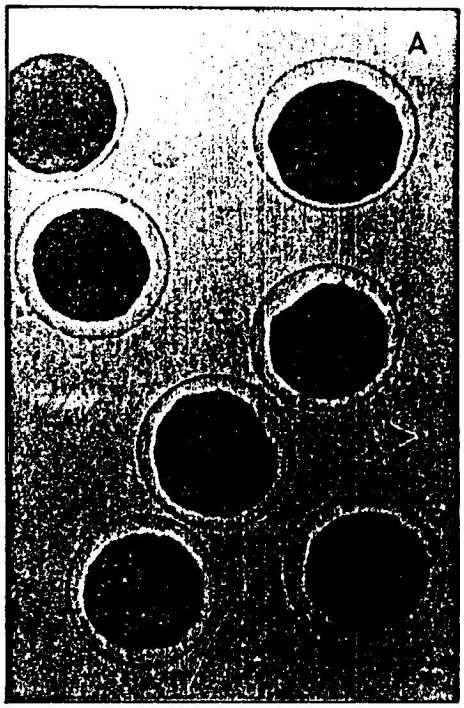
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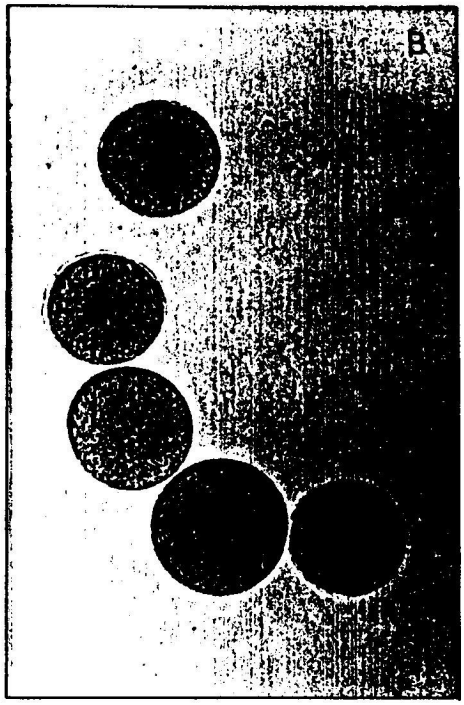
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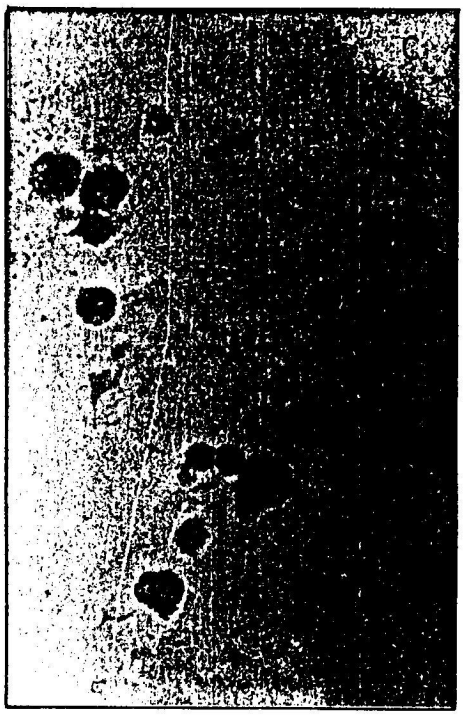
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