be due to the presence of saccharidic complexes. The common denominator of all these compounds is their acidophilia and their staining by Weigert's fibrin and P.A.S. methods. These reactions might be due to free amino-groups, many of which are probably in a 2 position in respect to hydroxyls (as the deamination procedures had some effect on the P.A.S. staining). It seems probable, although it has not been proved, that the complexes responsible for the staining reactions of the fibrinoids and fibrin might be different polysaccharides, possibly poly-amino-sugars (1). Our findings corroborate those of other workers who found that "fibrinoids" differed from fibrin in their histochemical(1,6,8,9), physical(10), and biochemical(11) characteristics.

The multiplicity of "fibrinoids" confirms and extends the views expressed by Klemperer(5) and by Gueft and Laufer(12) who maintained that the systemic Lupus erythematosus fibrinoid derives from DNA and is therefore chemically different from the other "fibrinoids."

Summary and conclusions. 1. The types of "fibrinoids" in different pathological processes were studied by means of specific staining reactions and histochemical procedures intended to block amino groups. 2. All fibrinoids differed from fibrin in their behavior. The "fibrin" layer around the placental villi behaved more like fibrin than any other "fibrinoids." The other "fibrinoids" differed markedly from each other and from fibrin. It may be concluded that in spite of their tinctorial similarities, the various "fibrinoids" contain different compounds and might represent the end-stages of different processes.

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Effect of High Hydrostatic Pressures on Colchicine-Damaged Mast Cells of Peritoneal Fluid.* (22467)

JACQUES PADAWER,† ARTHUR M. ZIMMERMAN, ALBERT S. GORDON, AND DOUGLAS MARSLAND.

Departments of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, and Biology, Graduate School of Arts and Science, N. Y. University.

Morphologic changes in the mast cells of rat peritoneal fluid have been induced by subcutaneous injections of colchicine(1). The cells, normally spherical, are altered into irregular masses, sometimes with branched pseudopod-like extensions of cytoplasm, and the nucleus appears to be pushed to the periphery of the granular cytoplasmic mass as if being ejected from the cell. These changes

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are apparent within 3 hours following injection of the drug. Since cells normally round up when suspended in a fluid medium, this unusual behavior of free-floating mast elements in the peritoneal fluid of colchicine-treated rats suggests possible alterations of sol-gel equilibrium within the cytoplasm. The application of high hydrostatic pressure has proved an excellent tool for the study of sol-gel equilibria in ameboid movement(2) and in dividing marine eggs(3). In the present study, high hydrostatic pressure technics have been employed to investigate whether the abnormal, non-spherical shape of mast cells in colchicine-treated rats is associated with a strongly gelated cytoplasm.

Materials and methods. Intact young adult female rats (150 ± 10 g) of a modified Long-Evans strain were given a single subcutaneous injection of freshly prepared colchicine alkaloid (Eimer and Amend, U.S.P. X11) solution in 0.9% NaCl (0.1 mg/100 g body weight). The animals were killed by exsanguination under light ether anesthesia 3-5 hours after the injection. Peritoneal fluid was obtained as described previously(4-6) and 1 volume aliquots were added to approximately 40 volumes of freshly obtained rat serum diluted 1:1 and 1:2 with 0.9% saline. The apparatus used for subjecting the cells to high hydrostatic pressures has been described(7). The experiments were carried out at 38 ± 1°C. The apparatus is designed to allow uninterrupted direct observation of the cells within the pressure chamber at a magnification of 480 diameters throughout the application of pressure. Specific cells within the microscope field were kept under constant direct observation for the duration of the experimental run. The cells were placed in the pressure chamber immediately upon removal from the peritoneal cavity of the animal and a new rat was used for each determination. A control aliquot of each sample was kept at atmospheric pressure in a hemacytometer for the duration of the experiment and specific mast cells were observed similarly.

Results. Hydrostatic pressures of 8,000 lbs/in^2 maintained for 20 minutes or 10,000 lbs/in^2 maintained for 5 minutes did not affect the morphology of the abnormal mast cells. However, with pressures of 12,000 lbs/in^2, some aberrant mast elements evidenced rounding up within 45 seconds of pressure application. Not all the abnormally shaped cells were affected. In general, forms departing moderately from the normally spherical or ovoid shape were responsive to the application of pressure while the more strikingly polymorphic mast elements appeared but slightly affected by the applied pressure. Some free-hand sketches of the changes observed are reproduced in Fig. 1. In some cases, cells which could not be induced to round up completely under pressure did so when the pressure was released instantaneously from 12,000 lbs/in^2 to atmospheric level. Under these circumstances, cells which had already assumed a spherical shape appeared to shrink slightly. All the control mast cells maintained at atmospheric pressure remained morphologically unaltered throughout the observation periods.

Discussion. Cells freely suspended in a liquid medium generally tend to assume a spherical shape. This rounding is usually attributed to tensional forces at the cell surface, which reach a minimum when the cell is spherical. Moreover, it presupposes that any gel structure in the cytoplasm is weak or absent and that there is no rigid pellicle.

In the peritoneal fluid of young rats, mast cells and other cellular types are normally spherical, whereas in the connective tissue spaces they are polymorphic. This suggests that the tensional forces and gelational states may be altered by contact with solid surfaces. Thus the cells become deformed in accordance with the principles described by Garber (8) and by Weiss and Garber(9) for fibroblasts in tissue culture.

The fact that the mast cells in the peritoneal fluid of colchicine treated animals display a high degree of polymorphism(1) suggests that the drug may have a definite effect upon the plasmagel structure of this cell. Apparently, it initiates the formation of strongly gelled localized cytoplasmic regions which can contract, forcing an outflow of plasmasol into

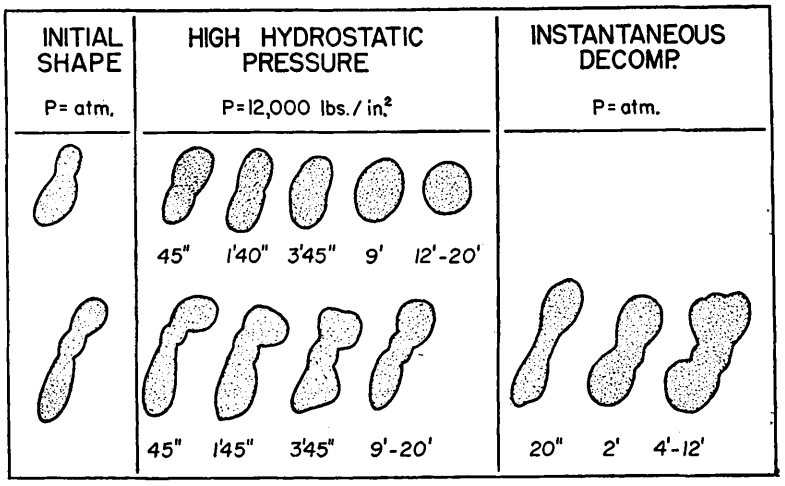

FIG. 1. Free hand sketches of mast cells visualized within the pressure chamber. Left: Optical cross section of 2 cells prior to application of pressure. Center: Consecutive sketches of these 2 cells while under pressure. Right: Changes observed after release of pressure. The upper cell is typical of "responsive" ones while the lower one represents the "unaffected" type. This difference arises presumably from different degrees of initial cytoplasmic gelation.

pseudopodium-like processes, in a manner analogous to ordinary amoeboid movement. This viewpoint, in fact, is well supported by the results of the present experiments. One well established effect of high pressure is to shift the sol-gel equilibrium, causing a drastic solation of the plasmagel system which would permit the cells to reassume their normal rounded form. It seems possible that the more highly aberrant forms are more firmly gelled since these do not become completely rounded even by pressures of 12,000 lbs/in^2. It is probable that even the most abnormally shaped mast cells would round up more completely if higher pressures were applied.

Sol-gel and pressure-volume relations of cells have been discussed in more detail in previous publications(2,10). The effect of colchicine on cytoplasmic viscosity has been investigated by Beams & Evans(11) for *Arbacia* eggs. They report a definite lowering of cytoplasmic viscosity at the interior of the cell with a probable, although less evident, effect at the cell surface. Colchicine does not inhibit locomotion in *Amoeba* or cyclosis in *Tradescantia*, both of which presumably depend on sol-gel relations(11). Wilbur(12) has suggested that colchicine affects cytoplasmic viscosity of *Arbacia* eggs indirectly "by inhibiting processes which do produce viscosity changes."

Mast cells are not generally considered to undergo mitosis. Therefore, the reasons for their sensitivity to colchicine both *in vivo*(1) and *in vitro*(13) as well as to podophyllotoxin (another mitotic inhibitor) under similar conditions(13) remain to be elucidated.

Summary and conclusions. Abnormally shaped mast cells of peritoneal fluid in colchicine-treated rats are affected by the application of high hydrostatic pressure. The changes observed under pressure include rounding up of the cell, suggesting that the abnormal morphology is maintained by a strongly gelated cytoplasm. Induction of localized foci of gelation by colchicine is postulated to explain the formation of polymorphic mast elements.

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Induced Accumulation of Citrate in Therapy of Experimental Lead Poisoning.* (22468)

JOAN F. FRIED, MARCIA WHITE ROSENTHAL, AND JACK SCHUBERT.
(Introduced by A. M. Brues.)
Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

Chelating or complexing agents are widely used to counteract the toxicity of metal ions and to accelerate the elimination of radioelements from the body. Since the distribution of administered water-soluble chelating agents is primarily extracellular it is unlikely that these chelating agents are in sustained contact with cellular components. For this reason we have explored the possibility of interfering with metabolic processes *in vivo* so that certain compounds which are naturally present in the body and which possess chelating or complexing properties might be maintained in abnormally high concentrations within the cells and thus be utilized to modify metal toxicity. Citric acid is a compound which is intimately involved in the Krebs cycle and which possesses complexing properties. Citrate ion, Cit^{3-}, forms a stable complex with Pb^{++} ($PbCit^{-}$) under physiological conditions; the logarithm of the formation constant for this reaction is 5.74(1). For these reasons, lead was chosen as the toxic agent and citrate as the metabolic chelating agent. Sodium citrate in clinical use has resulted in symptomatic relief in some cases of lead poisoning(2-4). However, it was necessary to administer large doses at frequent intervals over fairly long periods of time, because citrate ion, administered either orally or parenterally, disappears rapidly by excretory and metabolic pathways(5). These facts suggested that an induced maintenance of high citrate levels *in vivo* might be of greater therapeutic value than the repeated administration of exogenous citrate(6). Administration of sodium fluoroacetate (FA) in lethal doses has been shown by Peters *et al.*(7) to produce synthesis of fluorocitric acid which acts as a competitive inhibitor in the enzymatic destruction of citric acid in the tissues(8-10). It has been shown(6) that a single injection into rats of a small, non-lethal dose of FA causes a pronounced accumulation of citric acid in many tissues though not in blood. While the control levels of citrate in the kidney and spleen were roughly 20 μg and 70 μg per gram fresh tissue respectively, maximum levels of more than 10 times normal were reached in the kidney, spleen, and heart within 4 to 6 hours and high levels were maintained for several more hours.

In the present experiment the survival of animals given toxic doses of Pb^{++} ion and treated with small, sublethal amounts of fluoroacetate has been compared with that of citrate-treated and untreated animals.

Materials and methods. The animals used were 78- to 181-day-old Sprague-Dawley female rats weighing between 225 and 270 g. In each series of experiments animals were

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