which some of the 'fixed' sites are displaced by small molecular 'mobile' ligands, *e.g.*, H_2O, Cl^-, etc., and perhaps even metabolites such as pyruvate and citrate(4) passing in and out of the cell. Thus, during metabolism, the aggregate would be 'opened up' and the tissue should dissociate more readily. The facilitating effect of high pH on dissociation observed not only in liver, but in other tissues (5,6) may be explained in part similarly. Hydroxyl ion coordinates with cations and would diffuse in and open up the tissue by displacing some of the 'fixed' surface sites.

According to the coordination hypothesis of aggregation which we are considering, the primary event is the completion of the coordination requirements of the intercellularly held cation through the formation of one or another of the configurations 'permitted' for that cation. It is not necessary to distinguish between the cases in which cells are aggregated directly to other cell surfaces, and those in which there is intercellular matrix material. In both cases, the stability of the complex may be expected to be determined, and amplified, by secondary cooperative effects taking place along the membrane. The sensitivity of aggregation to pH and to temperature observed here and with other systems(7) may be the result not only of changes in metabolism but to conformational changes of the macromolecules involved.

Summary. 1. Dissociation of adult mouse liver *in vitro* was found to proceed more rapidly at 38° than at 4°C. The number of cells recovered either in the presence or the absence of TPB was increased. The physiological condition of the cells recovered by dissociation at 38°C was superior. 2. At 38°C, the concentration of TPB required for dissociation correlates well with the concentrations where it complexes K^+. 3. A procedure has been developed which results in the recovery of about 80% of the total population of parenchymal cells as a suspension of single intact cells.

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Role of 'Intercellular' Matrix in Aggregation of Mammalian Cells. (30954)

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Previous work(1) has shown that K^+ complexing agents can dissociate adult mouse liver into a suspension of single cells. On the basis of this finding, we have proposed a coordination model for aggregation of cells in tissues. In the course of investigating various aspects of this model, we have reinvestigated the dissociation of liver by acid phosphate, first reported by Langmuir and ap Rees(2). It has been found that dissociation in acid phosphate is inhibited by hydrocortisone. In the presence of low concentrations of hydrocortisone, long strands of an intercellular material not apparent under other conditions of dissociation, aggregate and trap the cells in an entangled mesh.

This finding helps to clarify some specific physical questions presented by the coordina-

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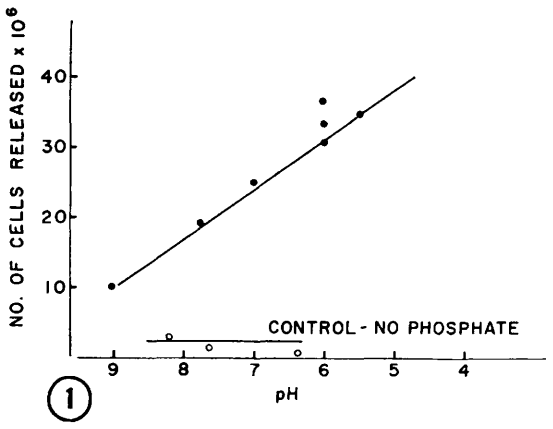

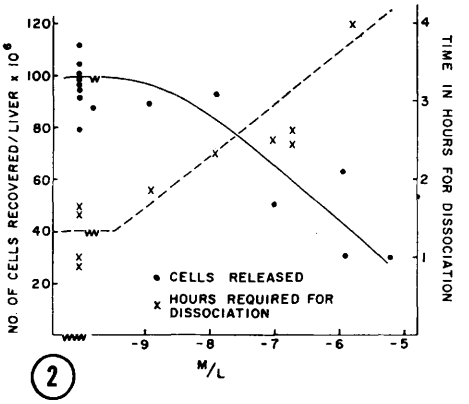

FIG. 1. Number of cells recovered on treatment of adult mouse liver by 0.1 M sodium phosphate buffers at different pH's.

FIG. 2. Inhibition by hydrocortisone of release of cells from liver during treatment with phosphate at pH 6.8.

tion model of aggregation.

Materials. Hydrocortisone was obtained as the 'hydrocortisone-alcohol micronized' from Chas. Pfizer and Co. It was kept as a 0.5% stock in absolute ethanol.

Results. The number of cells released from adult mouse liver treated *in vitro* at 4°C with 0.1 M sodium phosphate buffers is given in Fig. 1. In these experiments, liver had been cut into pieces 3-5 mm in size and preincubated for 2 hours at °C in a .05 M sucrose-.14 M NaCl solution, supplemented with 0.1 M phosphate at the indicated pH(see (1)). At the end of this incubation period, the tissue was 'reduced' by pipetting and the number of cells released determined by haemocytometer counts. The effect of phosphate increases with decreasing pH and at pH 5.5 about 35 million cells are recovered from an adult mouse liver.

Comparison of these results with previous data on TPB(1) shows that yields with acid phosphate are about 50% of those obtained with .003 M TPB at pH 8.6. The cells released by phosphate are also in a much poorer physiological condition than those released by TPB. The condition of the cells was not improved significantly by using phosphate dissociation solutions in which the concentration of NaCl had been reduced so that total concentration of Na^+ was within a physiological range.

Raising the temperature during treatment from 4°C to 38°C was found to increase the yield of cells liberated by phosphate. At 38° C and at pH 6.8 about 90 million cells could be recovered by treatment in the 0.1 M phosphate-sucrose salt solution. This represents 60-70% of the number of cells which can be recovered with TPB at 38°C. Furthermore, whereas the cells liberated with TPB survive and grow *in vitro*, the cells liberated with phosphate were not found to survive *in vitro*.

The effect of hydrocortisone on phosphate dissociation at pH 6.8 at 38°C is shown in Fig. 2. Concentrations as low as 10^{-7} M significantly retarded dissociation and higher concentrations were found to decrease the number of single cells which could be recovered. In these experiments, 0.3 g wet weight of cut liver was stirred on a magnetic stirrer with 25 ml of the .05 M sucrose-.14 M NaCl solution, buffered with 0.1 M sodium phosphate at pH 6.8. In the absence of hydrocortisone, the tissue is completely dispersed in about 2 hours. At 10^{-6} M hydrocortisone, the dispersion takes at least 4 hours, and with some livers, dispersion is completely inhibited, with very few cells liberated even after 24 hours.

The appearance of the suspensions in the presence and absence of hydrocortisone is shown by the photomicrographs in Fig. 3 and 4. In the phosphate control, the cells are seen to lie free in a solution containing red blood cells, nuclei and some cellular debris. In the presence of hydrocortisone at 10^{-7} M,

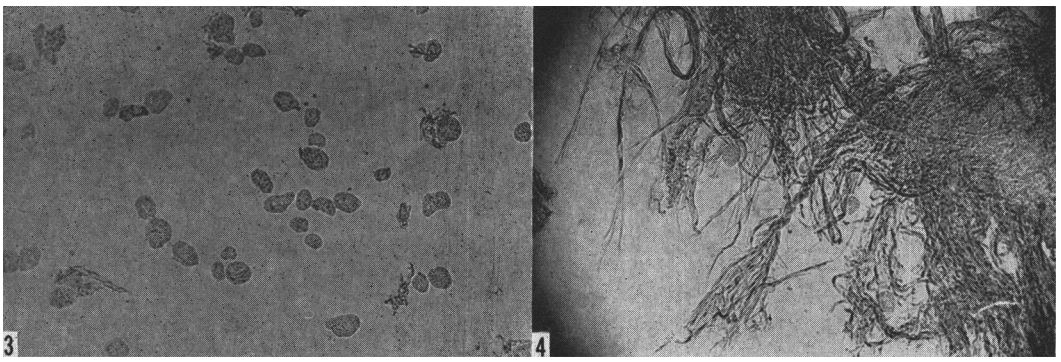

FIG. 3. Single cell suspensions recovered by treatment of mouse liver with 0.1 M sodium phosphate pH 6.8 at 38°C.

FIG. 4. Same liver as dissociated in Fig. 3 showing aggregation of intercellular material appearing during dissociation in the presence of 10^{-7} M hydrocortisone in the 0.1 M phosphate pH 6.8 solution.

a material forming long strands becomes visible at low power. The inhibition of dissociation is due largely to the trapping of cells in an entangled mass.

Hydrocortisone was found to have no significant effect on either the rate of dissociation or the number of cells which could be recovered when the tissue was treated at 4°C. The suspensions prepared at 4°C with hydrocortisone were also free of any fibrous material.

Discussion. The macromolecular material which has been made visible by the presence of hydrocortisone during dissociation at 38° C by acid phosphate appears to be the 'intercellular matrix.' Fixation and staining of liver sections has shown the presence of an intercellular 'reticulin.' During dissociation, where viable cells are separating from each other, the material can be seen to be situated between the cells. The material, however, is not apparent when the dissociation proceeds in the cold. Temperature sensitivity on the state of aggregation has also been found to be a striking characteristic of another and better studied class of intercellular materials, i.e., the collagens(3).

The finding that a significant number of the cellular aggregates in liver tissue involve an intercellular component indicates a possible mechanism for dissociation that does not involve removal of K^+. Any condition which would tend to destabilize this material would cause some breakdown of the tissue structure. These conditions may include critical ionic strengths and pH of the salt solutions used for dissociation, and the relative concentration of cations, e.g., Ca^{2+}, Mg^{2+} or Na^+ which may be involved in the stabilization of aggregates of these materials. The partial dissociation of liver by acid phosphate, as well as by high concentrations of EDTA(2), or by trypsin are considered to be the result of 'destabilization' or digestion of the intercellular component. The facilitating effect of increased temperature and pH on dissociation may be the result not only of metabolism, as previously discussed, but in part to a favorable conformational change in the intercellular matrix. This may also explain why preliminary perfusion of liver *in vivo* with agents such as polyvinylpyrrolidone, as in the classical preparation of liver cells(4), facilitates the dissociation of the tissue when subsequently treated *in vitro*.

Dissociation by agents acting primarily on the stability of the intercellular matrix has not been found to be as effective as the removal of K^+ with sodium tetraphenylboron. The findings indicate that the most efficient dissociation of a given tissue will be effected by the use of the appropriate monovalent cation complexing agent under conditions supporting metabolism of the cells and destabilization of any intercellular material.

The presence of an intercellular component in liver cell aggregates, as in many other systems studied(5) helps elucidate 3 specific

problems raised by the coordination model for aggregation which has been proposed. Coordination requires that the apposing surfaces are within 15A—and probably closer to 5A—at the coordination sites. It is difficult, with evidence now available, to evaluate distances between cells in cell-cell aggregates, but it seems unlikely that large areas of the surfaces are juxtaposed this closely. However, in cell-matrix-cell aggregates, the restriction on spacing of cells is eliminated. The hypothesis also requires that water of hydration on monovalent cations can be displaced by other ligands, both 'fixed' on the cell surface and 'mobile.' A variety of studies(6,7), have now indicated that the structure of water is ordered in the vicinity of these macromolecules and this ordering may be such as to restrict hydration of surface-bound cations. The coordination hypothesis has explicitly stated that there is a coupling between the state of aggregation of cells and electrolyte exchange. In this process, highly conformable macromolecules situated at the cell surface which orient water and other electrolytes (8,9) may be expected to play a significant role.

Summary. 1. Partial and slow dissociation of mouse liver *in vitro* that occurs on treatment with phosphate indicates a K^+ independent factor involved in aggregation of cells. 2. The presence of low concentrations of hydrocortisone during dissociation in phosphate causes the aggregation into long fibers of an intercellular material. 3. Dissociation effected with phosphate has been attributed to a 'destabilization' of the intercellular matrix.

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Effects of Hyperbaric Oxygenation on Lactic Dehydrogenase Isoenzymes in Rats in Noble-Collip Drum Shock.* (30955)

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Inasmuch as dehydrogenases are affected in the brains of shocked rats(1) and hyperbaric oxygenation (OHP) increases survival after challenge in the Noble-Collip drum(2), it appeared of interest to study the behavior of dehydrogenases in different organs of the rat in shock and in OHP.

This report compares electropherograms of isoenzymes of lactic dehydrogenase (LDH) from brain, heart, kidney, liver, mucle (quadriceps) and plasma of the rat under the conditions mentioned.

Materials and methods. Young adult male Wistar rats weighing 190 to 250 g were used. Shock was produced by 640 turns in the Noble-Collip drum(3), with the fore and hind limbs of the rats fettered with adhesive tape. They were unfettered immediately after drumming and while one group was left in room air, another group was placed in a hyperbaric tank, a horizontal cylinder, 65 cm long, inner diameter 20 cm.‡

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‡ Built by Dixie Manufacturing Co., Baltimore, Md.