crease in the ΔA-values was also observed in aqueous solutions with increasing concentrations of alcohols (figure 2).

The difference spectra elicited upon interaction of CPZ with lipids and fatty acids have been measured using individual fatty acids as aqueous anionic solutions. The results obtained with saturated fatty acids are summarized in table 1, the ones with unsaturated fatty acids in table 2.

In the presence of urea (8 M), a chaotropic agent, the difference spectrum elicited by CPZ (50 µM) in the presence of 18.1-cis- or 18.1-trans fatty acid (10^{-4} M) disappeared. This was in contrast to the antichaotropic ion, fluoride, which produced a 1.6fold increase in ΔA of CPZ in the presence of 18.1-trans fatty acid (10^{-4} M). Finally, the ΔA-value of CPZ in hexane (see figure 1) was not increased by the addition to the hexane solution of palmitic acid (16.0) even at a concentration of 10^{-2} M.

Discussion. The difference spectrum of CPZ was first observed with serum albumin[5] and was believed to express hydrophobic interactions. This was also concluded from analogous studies with lipids which are biologically important binders of the drug[2,3] and which elicit the difference spectrum at much lower concentrations[4]. CPZ can undergo self-association[6,7] which also results in the appearance of the difference spectrum[4].

The appearance of the difference spectrum of CPZ with alcohols, nonpolar solvents, and fatty acids, as well as the increase of ΔA with increasing hydrophobicity of the environment adds more evidence to this difference spectrum representing hydrophobic interactions. In the case of nonpolar solvents, self-association of CPZ can be excluded for thermodynamic reasons[8].

Additional problems are exposed by the experiments with fatty acids (tables 1 and 2). The critical micellar concentrations of the fatty acids[9] are likely to be about one order of magnitude higher than the concentrations at which the difference spectrum appeared, indicating that interaction occurs with both micellar and monomeric fatty acids. One or more double bonds introduced into a fatty acid produce an upsurge in ΔA without appreciable changes in hydrophobicity. Since the ΔA of the cis- and trans-isomers of a fatty acid may vary considerably, steric rather than electronic factors must be assumed to influence the interaction.

The difference spectrum was abolished by urea (8 M) which is known to dissociate hydrogen bonds and thus hydrophobic interactions[8,10]. The difference spectrum of CPZ with serum albumin or microsomal fractions was also abolished by urea, and this was paralleled by a decrease in their binding as determined with equilibrium dialysis[3,4]. In contrast, fluoride ions, which enhance hydrophobic interactions, increased ΔA.

In conclusion, the binding of CPZ to fatty acids is mainly due to hydrophobic interactions; however, steric factors of the fatty acids play an additional role in the magnitude of the interaction spectrum and thus possibly in the actual binding process.

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Erythrocytes within pancreatic B-cells of corticosteroid-treated mice

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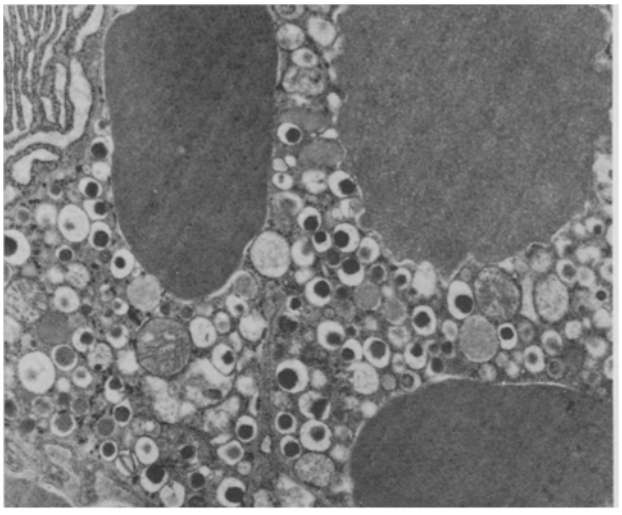
Summary. An ultrastructural study of the endocrine pancreas of female ICR mice that received 21 daily injections of the synthetic glucocorticoid, Triamcinolone diacetate (8 mg/kg b.wt) revealed some examples of microhaemorrhage within islets of Langerhans, extravasation of erythrocytes, and the presence of erythrocytes within B-cells, where they undergo degradation to form myelin-like configurations.

Glucocorticoids are generally regarded as potential diabetogenic agents in both experimental animals and man[1-6]. During the course of prolonged administration to mice of a synthetic glucocorticoid, Triamcinolone diacetate, an analogue of cortisol, followed by an ultrastructural examination of their Islets of Langerhans, we have encountered some instances of erythrocytes apparently within B-cells. So far as we are aware, this is the first recorded report of such erythrocytic extravasation and we would like to record briefly the details.

Material and methods. Female ICR mice, 6 weeks old, received 21 daily s.c. injections of 8 mg/kg b.wt Triamcinolone diacetate ('Ledercort', Lederle Labs, American Cyanamid Co., New York). Untreated littermates maintained under identical conditions served as controls. All animals were maintained on mouse Purina chow (Ambar Purina Co. No. 19-210, Israel) and tapwater ad libitum. During the final 20 h before sacrifice, only water was made available. Mice were killed by neck dislocation and their pancreases carefully removed and immersed in ice-cold (4 °C) 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Pancreases in the fixative were cut into small blocks with ultrafine dissection scissors under a binocular microscope, by means of which the islets are readily visible, and fixed for 2 h. Blocks were stored overnight in the cacodylate buffer containing 7.5% w/v sucrose, postfixed in 1% osmium tetroxide, dehydrated in graded concentrations of ethanol and embedded in Epon 812. Epon sections, 0.5 µm thick, were stained with 0.1% toluidine blue in 1% borax for light microscopy. Sections, 60–90 nm thick, of selected blocks containing islets, were cut with glass knives on a Cambridge-Huxley Mk 1 ultramicrotome, collected on un-



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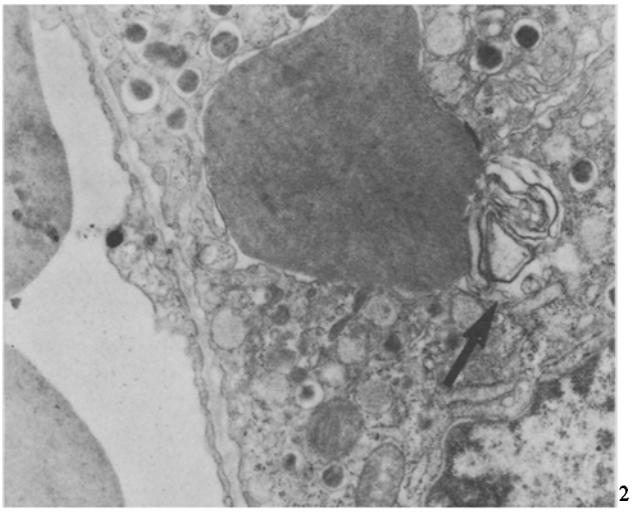

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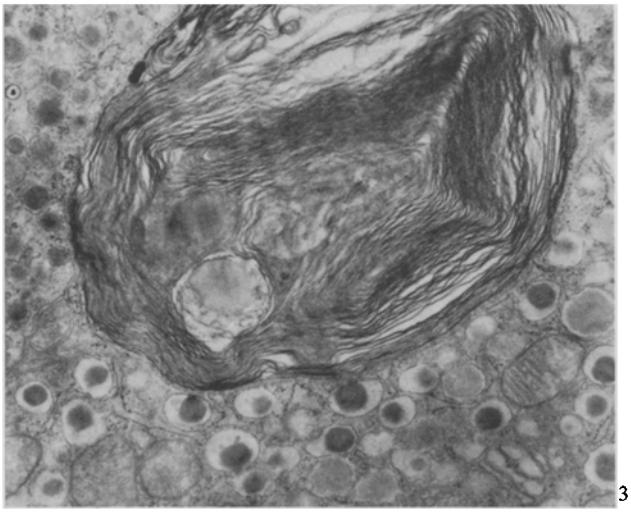

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Figs. 1-3. Portions of endocrine pancreas of Triamcinolone-treated mice. Fig. 1. Erythrocytes seen within B-cell. × 6000. Fig. 2. Erythrocyte in B-cell with partial development of membranous configuration (arrowed). Note erythrocytes in adjacent capillary bounded by intact endothelial cell wall. × 10,000. Fig. 3. Complex membranous configuration formed from degraded erythrocyte in B-cell. Part of A cell is seen in upper left of micrograph. × 10,000.

coated copper grids and stained briefly in aqueous uranyl acetate and lead citrate. Electron micrographs were taken with a JEOL 100B electron microscope at 80 kV.

Results. In 3 treated mice we found localized microhaemorrhage within islets of Langerhans. Erythrocytes were present in both extravascular sites between the endocrine cells of the islets as well as within the cytoplasm of B-cells (figure 1).

Several cases were found showing the appearent degradation of erythrocytes within B-cells and the subsequent formation of myelin-like configurations (figures 2 and 3). Some fairly dense rounded granular bodies, reaching 3 µm in diameter, were occasionally present in these cells and possibly represent a condensation product of the contents of erythrocytes or a stage in their lysis.

Erythrocytes were not found within the other endocrine cells of the islets, nor within acinar cells of the exocrine pancreas of treated mice[7]. Similarly erythrocytes were absent from pancreatic cells of control mice.

Discussion. It is well known that glucocorticoids affect the vascular system, usually causing vasoconstriction and decreased extravasation of both cells and fluid[8]. We have, on the contrary, found many cases of internal haemorrhage in mice chronically treated with pharmacological doses of Triamcinolone (unpublished observations). Spontaneous haemorrhage within hyperplastic pancreatic islets associated with glycosuria has been recorded in hybrid mice[9].

Whereas we do not entirely rule out the possibility that the mode of handling or specimen preparation may have caused the erythrocytic extravasation, no damage was seen in the walls of capillaries in the area of the islets. Moreover, no cases of erythrocytes in extracapillary sites were found in control mice. In rats, microhaemorrhage and erythrocyte uptake by thyroid epithelial cells resulting from goitrogen treatment has been shown to be independent of the mode of fixation or specimen preparation[10].

The mechanism of erythrocyte penetration into B-cells or their engulfment is unresolved. Erythrophagocytosis has now been reported in a variety of cells not normally considered to be phagocytes under pathological or pharmacological conditions[10,11]. The intracellular degradation of erythrocytes and subsequent formation of myelin-like configurations is similar to that recently found in thyroid epithelial cells in vivo[12].What is not clear is why erythrocyte uptake in our mice was restricted to B-cells or why this phenomenon was only found in specific individuals. A previous case of erythrophagocytosis also showed marked response specificity of the individual[13].

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