the more alkaline pH that may be experienced at the lateral cell surface when IgG is diacytosed. Such a simple explanation cannot hold for all IgG transporting epithelia since endodermal cells from rabbit yolk sac, exposed to the more neutral pH conditions of the uterine fluid, show little if any pH dependency for EA rosette formation between 6.0 and 8.0[15] and similar findings have been reported for binding of IgG to the Fc receptor on human syncytiotrophoblast exposed to maternal blood[16]. However, the Fc dependent binding of sensitized SRBC to the lateral and basal surfaces of proximal gut enterocytes indicates that receptor sites are regenerated here. We would favour the interpretation that these arise from coated vesicles that have previously selectively endocytosed IgG at the apical cell surface and then moved through the cell and subsequently fused with the lateral and basal plasmalemma so discharging their contents. Evidence for such a process has been obtained both in suckling rat gut[2,9] and rabbit yolk sac endoderm[17] at the ultrastructural level. The disappearance of Fc receptors (as indicated by decrease in EA-enterocyte rosette formation) as the young rat ages, correlates precisely with what is known about decrease and cessation of antibody transport to the blood of the suckling rat, and is in accord with studies of binding of ^{125}I-labelled mouse monomeric IgG by jejunal enterocytes of suckling rat gut as revealed by autoradiography[4]. In the latter studies[4] evidence has also been obtained that Fc receptors on jejunal enterocytes are trypsin-sensitive and restricted to those cells on the more apical regions of villi. This would explain why not all the enterocytes removed from proximal gut segments formed rosettes and why wide variations occurred in the number that did form rosettes when different rats at 12 days postpartum were compared.

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Shape change reaction of platelets in protein-free medium: ultramorphology

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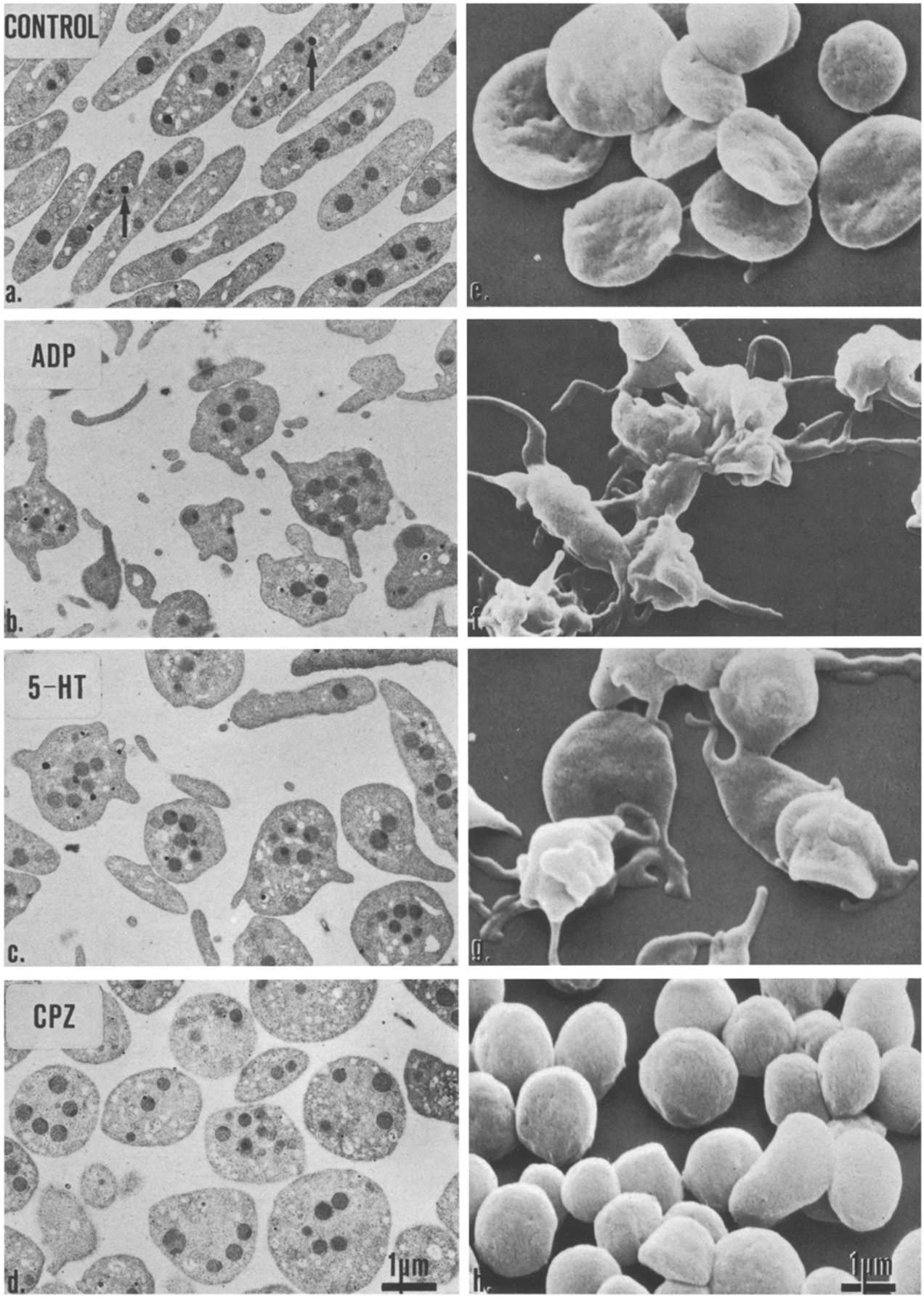
Summary. Transmission and scanning electron microscopy indicate that rabbit platelets incubated in protein-poor medium retain their reactivity to shape change-inducing agents such as 5-hydroxytryptamine, adenosine-5′-diphosphate and chlorpromazine. Such platelets may be used as models for drug-membrane interactions, e.g. in neuronal cells.

During the shape-change, reaction blood platelets undergo a transformation from their normally discoid form into a spheroid shape, whereby the light absorption of the platelet suspensions is increased[1]. The shape-change reaction, i.e. the increase in light absorption, has generally been measured using plasma-containing media, which may however introduce sources of error (see below). Therefore, a method has been developed which allows the suspension of the isolated platelets in protein-free buffer without greatly impairing their reactivity to shape change-inducing agents such as 5-hydroxytryptamine (5HT), adenosine-5′-diphosphate (ADP) and chlorpromazine (CPZ)[2,3]. This paper describes the effects of these compounds on the ultramorphology of isolated platelets in protein-poor medium.

Materials and methods. Platelets of rabbits, isolated by centrifugation of platelet-rich plasma (PRP) on a discontinuous dextran T-10 gradient, were resuspended in about 4 vol. of citrate buffer containing glucose and saline, pH 7.4, 290 mosmol, as previously described[2]. After incubation in glass tubes for 30 min at 37°C, the platelets were stirred for 2 min at 37°C with a teflon-covered stirrer at 900 rpm. 5HT-creatinine sulfate (final concentration 5×10^{-5} M), ADP (disodium salt, 5×10^{-5} M), CPZ (hydrochloride 10^{-4} M) or solvent alone (H_2O) were then added[2,3]. When the light absorption had reached its maximum, i.e. after 60 sec (5HT), 30 sec (ADP) and 5 min (CPZ) an equal volume of 0.2% glutaraldehyde (in 0.1 M cacodylate buffer pH 7.3) was added and the platelets were prefixed for 30 min at room temperature. Thereafter, each specimen was divided into 2 parts and processed for transmission[2] and scanning[4] electron microscopy (TEM and SEM).

Results and discussion. With both TEM and SEM, the control platelets showed a discoid shape and the outline of the plasma membrane was smooth, except for occasional

Transmission (left) and scanning (right) electron micrographs of rabbit blood platelets isolated on a dextran gradient, resuspended in protein-free buffer and exposed to various compounds. Note the discoid shape of control platelets, the shape change and pseudopods induced by ADP and 5HT and the shape change with virtually no pseudopods after CPZ. Arrows identify highly electron-dense organelles.


CONTROL
a.
e.
ADP
b.
f.
5-HT
c.
g.
CPZ
d.
1µm
h.
1µm

pits and craters (figure). The cytoplasmic organelles, such as a- and 5HT-granules, were randomly distributed and the microcanalicular system was not dilated. In contrast, the majority of the ADP- and 5HT-treated platelets had a spherical, often irregular form with extrusion of pseudopods or blebs, these changes being more pronounced in ADP-treated platelets. The subcellular organelles and the microcanalicular system were, however, not markedly changed. The platelets exposed to CPZ were generally spheroid without extrusion of pseudopods or blebs. The subcellular organelles and canaliculi seemed to be virtually normal, except for the highly electron-dense 5HT organelles which had either totally or partially lost their osmiophilic content (figure).

These changes are similar to those occurring when platelets incubated in plasma are exposed to 5 HT, ADP or CPZ[1,4-12]. Also it has been demonstrated that, in platelets treated with 5HT and ADP, the plasma volume trapped between the centrifuged platelets is increased[1]. Preliminary experiments with suspensions of rabbit platelets in protein-free buffer gave similar results. In fact, in sections of platelet pellets (obtained from the same platelet pool under identical conditions of centrifugation) the number of platelets per square unit was 131 and 178 in ADP-platelets and 206 and 225 in 5HT-platelets compared with 375 and 388 in the controls (2 experiments).

Measurements of light transmission have previously shown that rabbit platelets isolated by a dextran gradient and incubated in protein-free buffer retain their reactivity to shape change-inducing agents[2]. The electron microscopic findings now confirm these results. The use of protein-free buffer as an incubation medium is of considerable interest, e.g. in pharmacological experiments in which the 5HT receptors of the platelet membrane are taken as models for the neuronal 5HT-receptors of certain areas of the central nervous system[3]. In fact, the potency of drugs has been shown to be higher in protein-poor medium than in plasma, probably due to protein-binding in the latter[2].

The present results confirm that agents which interfere with different receptors of the plasma membrane (e.g. 5HT and ADP receptors)[1,3,13] may cause morphologically similar shape change responses. On the other hand, there is evidence that the platelet shape change induced by CPZ is morphologically different. This shape change may be due to unspecific platelet damage, as indicated by the loss of osmiophilic material from the 5HT-organelles and by previous ultrastructural[9] and biochemical[14] investigations.

In conclusion, platelets suspended in protein-poor medium seem to be easily accessible models for testing the effects of drugs on membranes in physiological and pathophysiological states.

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Thymectomy modifies androgenizing effects of a testis transplant during critical period for neuroprogramming[1]

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Summary. Thymectomy simultaneous with transplantation of a syngeneic testis from a littermate to Fischer 344 rats ameliorated the androgenizing effects of the testis transplant on ovarian morphology at 90 days of age.

Pierpaoli et al.[2] have suggested that the thymus gland is involved in the process of neuroprogramming during critical periods of pre- and early postnatal periods in rodents. Gorski[3] has shown that transplantation of a syngeneic testis during critical post-natal periods in rodents leads to eventual development of anovulation. The present investigation was undertaken to determine if thymectomy had an effect on the outcome of this classical demonstration of neuroprogramming.

Fischer 344 female rat pups were anesthetized with ether and thymectomized or sham-thymectomized at 5 days of age using a modification of the technique of Hard[4]. At the time of surgery, alternate littermates were implanted s.c. in the neck over the area of the jugular vein with one testis from a littermate. At 90 days of age, all animals were sacrificed by decapitation, and the ovaries removed, weighed and fixed in 10% buffered formalin. Examination of the transplantation site in those animals receiving the transplant revealed that 100% of the rats in both groups accepted the graft. Weights (mean$\pm$SD) of the implanted testes for the sham-operated group were 187.3$\pm$93.5 mg, and for the thymectomized group were 127.1$\pm$36.7 mg. Histologically, all testes contained recognizable seminiferous tubules and interstitial tissue.

From each rat, 2 or 3 H & E stained, coded sections of constant thickness and approximately 10 unmounted sections apart obtained from close to the midline of the ovary were selected for study. These sections were photographed at low power and printed at constant magnification. The photographs were then scored for the number of follicles with 1 diameter greater than 350 μm[5], and the number of corpora lutea were recorded. This information was decoded, summarized, and is presented in the figure. No differences were noted in the numbers of follicles greater than 350 μm in any of the groups, although greater variation was noted in those groups receiving the testis trans-