envisaged, provided that the technological and physical problems are properly solved[13].

Riassunto. Due polimeri reticolati di nuova sintesi, contenenti gruppi amminici terziari, sono in grado di assorbire selettivamente eparina dal sangue. Essi non sono emolitici e non interferiscono con i fattori della coagulazione.

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The in vitro Effects of Several Progestogens, Estrogens and Non-Steroidal Compounds with Estrogenic Activity on Adenosine Diphosphate Induced Guinea-Pig Platelet Aggregation

The increased use of oral contraceptives appears to be associated with a statistically significant increase in the incidence of tromboembolic disease[1,2]. Because platelets occupy a central role in thrombus formation[3], many investigators have directed their attention towards the in vivo effects of oral contraceptive steroids on various aspects of platelet behavior[3] (e. g. platelet aggregation, adhesion and electrophoretic mobility). For example, ADP-induced aggregation of blood platelets is increased after 1 week treatment with estrogens alone[4] but only after treatment for 4 or more menstrual cycles with combination oral contraceptives[5,6]. Although investigators have reported that progestins produced no change in platelet aggregation[6,7], a recent long term study showed that after 2 years treatment with chlormadinone acetate aggregation was significantly accelerated[8].

The present study is the first report on the in vitro effect of steroids with estrogenic and progestonic activity on platelet aggregation.

Experimental methods. Blood was collected from female Syrian Random strain guinea-pigs and the platelet rich plasma (PRP) was obtained as previously described[9,10]. PRP was diluted to the desired platelet number with modified Tyrodes solution (Ca^{++}, Mg^{++}-free) pH 7.4 [composition per litre: 9.0 g NaCl, 0.2 g KCl, 1.0 g dextrose, 0.05 g NaH_2PO_4 and 1.45 g *Tris*[11]].

All test compounds were prepared as 30 mM aqueous dispersions by homogenizing in modified Tyrodes solution and stored at 0–5 °C.

Platelet aggregation was followed at 37 °C by employing a modification of the turbidometric method of BORN[12] as previously described[9,10,13,14]. Both 'sample' and 'reference' cuvettes in the Beckman DBG spectrophotometer contained PRP (0.4–0.6 ml) diluted with modified Tyrodes to a total volume of 2.25 ml and the recorder was arbitrarily set at 100% transmission. The test compounds were preincubated for 10 min by adding 0.25 ml of the aqueous dispersion to the 'sample' cuvette and 0.25 ml of modified Tyrodes to the 'reference' cuvette to compensate for volume changes. The final volume in each cuvette was 2.5 ml with a final platelet concentration of 150,000 platelets/cu/mm. Addition of the test compound (final concentration of $3 \times 10^{-3} M$) caused an instantaneous drop in the % transmission. At the end of the preincubation period 0.25 ml of $1 \times 10^{-5} M$ ADP (final concentration of $9.1 \times 10^{-7} M$) was added to the 'sample' cuvette and 0.25 ml of modified Tyrodes to the 'reference' cuvette and changes in light transmission were monitored. Appropriate controls were obtained be measuring the degree of platelet aggregation caused by a similar concentration of ADP in the absence of any added test compound. Aggregation is defined as the change in the % transmission after the initial instantaneous drop in light transmission due to platelet swelling or from the time of ADP addition when swelling did not occur.

Results and discussion. Because all the drugs used in this study were virtually water insoluble and because most of the organic solvents in which these compounds are soluble have an effect on platelet swelling and aggregation induced by ADP, aqueous dispersions of the various drugs were used. The use of dispersions in the present study is not unique. For example the effect of insoluble agents

Effect of several drugs on ADP-induced platelet aggregation

Drug	Effect of ADP-induced aggregation
Norethindrone	+[a]
Medroxyprogesterone Acetate	+
Estrone	+
Ethinyl Estradiol	+
Mestranol	+
Diethyl Stilbestrol	−[b]
Hexestrol	−

[a] Stimulation of aggregation.
[b] Inhibition of aggregation. Incubation conditions and concentrations as described in experimental methods.

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such as collagen[15] and fatty acids[16] on platelet aggregation have been extensively studied in vitro.

The results of the present investigation are shown in the Table. Both the progestogens (medroxyprogesterone acetate, norethindrone) and estrogens (ethinyl estradiol, mestranol, estrone) stimulated ADP-induced aggregation over the first 2 or 3 min. Similar results were obtained with PRP samples taken from more than 6 different animals. None of the test compounds caused significant aggregation themselves during the preincubation period. In many instances the progestogens caused a slightly greater stimulation of ADP-induced aggregation than did the estrogens.

It should be noted that the steroid suspensions used in this study are not found in vivo, whereas fatty acids and especially collagen are encountered in vivo as suspensions or fixed material. Therefore, although the in vitro action can be due to the steroid configuration of the suspended material, the present results cannot be extrapolated to the in vivo situation.

In order to investigate the possibility that stimulation of ADP-induced aggregation might be due to platelets adhering to the particles in suspension and/or the biological activity of the test agent, the effects of diethylstilbestrol and hexestrol (2 water insoluble non-steroids with strong estrogenic activity) on platelet aggregation were tested. Both drugs caused an inhibition of ADP-induced aggregation thus suggesting that in the present experimental in vitro system: a) stimulation of aggregation was due to the steroid itself and was not a non-specific adhesion of platelets to the suspended material, and b) the ability to stimulate aggregation was related to the presence of all or part of the steroid structure and not the biological activity of the drugs in question (i. e. estrogenic activity).

Résumé. Des dispersions aqueuses de progestogènes et estrogènes stimulent l'agrégation des plaquettes de cobaye produites par ADP in vitro. Le diéthylstilbestérol et l'hexestrol qui ne sont pas des stéroïdes, mais possèdent une activité estrogénique, ont un effet inhibitoire sur l'agrégation.

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Production of Common Enterobacterial Antigen by Members of the Family Enterobacteriaceae

Ten years ago KUNIN et al.[1,2] described a previously overlooked antigen produced by various members of the family Enterobacteriaceae. The antigen was discovered by means of the passive hemagglutination test using *E. coli* O14 antiserum. Since that time it has been shown that this antigen is produced in 2 different states: *E. coli* O14 and a few other strains of enteric bacteria, upon i.v. injection into rabbits, engender CA antibodies, the immunizing antigen being ethanol-insoluble[3,4]. In contrast, most other strains of Enterobacteriaceae do not induce the formation of CA antibodies in high titers but do prime rabbits specifically for a secondary response upon injection of subeffective amounts of immunogenic CA. The antigen produced by these strains is ethanol-soluble[5,6]. If the production of CA were a regular property of, and restricted to, the members of the family Enterobacteriaceae, then, this characteristic could be used as one of many criteria for taxonomic and diagnostic purposes. Indeed, CA antiserum, rather than numerous group or type-specific antisera, was utilized to identify an organism as a member of this family, for example, in kidney tissue of patients with pyelonephritis[7–9]. The possible usefulness of documenting CA production for taxonomic purposes is illustrated by the observation that pigmented *Serratia* belonging to the family Enterobacteriaceae do produce the antigen in contrast to pigmented strains of Flavobacterium, which do not belong to this group. However, a systematic study of the production of CA by members of the family Enterobacteriaceae has not yet been reported. Therefore, the present investigation was undertaken to determine whether all recognized genera and most species of Enterobacteriaceae do, in fact, produce CA.

To demonstrate CA the previously described procedures[10], namely, passive hemagglutination, passive hemolysis, and hemagglutination-inhibition tests were used. The strains were grown on brain veal agar in Kolle flasks, and the growth obtained after incubation at 37 °C for 18 h was suspended in phosphate hemagglutination buffer (Difco; pH 7.3) and heated at 100 °C for 1 h. The suspension was then centrifuged at 23,500 g for 20 min and the supernates were used as antigen to modify rabbit or sheep erythrocytes. Red blood cells (2.5% suspension), after 3 washings with the above buffer, were mixed with the antigens in a dilution of 1:10; the mixtures were incubated at 37 °C for 30 min; and the red blood cells were again washed 3 times to remove unattached antigen. Anti-CA sera were prepared by immunization of rabbits with semi-purified ethanol-soluble CA obtained from several sources (*E. coli* O7, *S. minnesota*, *S. dysenteriae* type 1) and by immunization with *E. coli* O14. Antibody titers ranged from 1:3200 to 1:6400. To demonstrate CA produced by the unknown strains, the hemagglutination test was used as follows. CA antisera, in dilutions to give strong hemagglutination with standard CA, was mixed with an equal volume (0.2 ml) of antigenically modified rabbit erythrocytes (0.2 ml) in the hemagglutination test. The mixtures were incubated at 37 °C for 30 min and the hemagglutination was read after centrifugation at 1300 g

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