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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CA[a] production by Enterobacteriaceae

Family	Tribes	Genera and species	CA production as determined by Hemagglutination	CA production as determined by Hemolysis and hemagglutination-inhibition
Entero-bacteriaceae (144)	*Escherichieae* (27)	*Escherichiae*	+ (17)[b]	+ (17)
		Shigella (Grs A, B, C, D)	+ (10)	+ (7)
	Edwardsielleae (10)	*Edwardsiella*	+ (10)	+ (2)
	Salmonelleae (33)	*Salmonella* (Grs A, B, C_1, C_2, D, E, L)	+ (12)	+ (12)
		Arizona	+ (12)	+ (2)
		Citrobacter	+ (9)	+ (2)
	Klebsielleae (50)	*Klebsiella*	+ (5)	+ (2)
		Enterobacter	+ (33)	
		E. cloacae		+ (2)
		E. aerogenes		+ (2)
		E. hafniae		+ (2)
		E. liquefaciens		+ (2)
		Serratia	+ (7)	+ (7)
		Pectobacterium	+ (5)	+ (2)
	Proteeae (24)	*Proteus*	+ (13)	
		P. vulgaris		+ (2)
		P. mirabilis		+ (2)
		P. morganii		+ (1)
		P. retgeri		+ (2)
		Providencia	+ (11)	
		P. alcalifaciens		+ (2)
		P. stuartii		+ (2)

[a] CA = common antigen of Enterobacteriaceae; [b] ( ) = number of strains tested.

for 2 min. For the passive hemolysis test, sheep erythrocytes, instead of rabbit red blood cells, were used and complement (Carworth Laboratories, Inc., New City, New York) in a dilution of 1:20 (0.1 ml) was added to the mixtures of antigenically modified red blood cells and CA antiserum. Hemolysis was read after incubation at 37 °C for 1 h. For the hemagglutination-inhibition test, antigen from the strains under investigation (0.2 ml of supernate) was mixed with 2 minimal hemagglutinating units of CA antisera and the mixtures were incubated for 30 min at 37 °C. Antigenically modified rabbit erythrocytes were then added and the hemagglutination test was completed as described above. Inhibition of hemagglutination was considered to be evidence of the presence of CA in the test antigens.

A total of 144 smooth strains of Enterobacteriaceae, obtained from CDC and from this laboratory, was tested for the production of CA, and the results are summarized in the Table. It may be seen that all 144 strains, representing 5 tribes and 12 genera, produced CA, as determined by the passive hemagglutination test. 72 of these strains were also studied by the other serological procedures, namely, passive hemolysis and hemagglutination-inhibition tests. Without exception these methods confirmed the presence of CA in these cultures. Therefore, it may be concluded that smooth strains of Enterobacteriaceae characteristically produce CA. It must be emphasized, however, that certain rough strains of Enterobacteriaceae fail to synthesize this antigenic determinant, and studies on the genetic control of the formation of this antigen are in progress in the laboratory of Dr. H. Mayer, Max-Planck-Institut für Immunologie, Germany. Since documentation of the production of CA may conceivably be utilized as one of many criteria to identify members of the family Enterobacteriaceae, further studies are needed to determine the formation of this antigenic determinant by other families and particularly by poorly characterized species that may or may not belong to the family Enterobacteriaceae.

Zusammenfassung. Nachweis eines allen Enterobakterien gemeinsamen Antigens. Sämtliche Stämme, bestehend aus 5 Familien und 12 Genera, erzeugten das Antigen, welches durch passive Haemagglutination, passive Haemolyse und Haemagglutinations-Hemmungsreaktion nachgewiesen werden konnte.

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[11] Study supported by Research Grant No. AI00658 from the National Institute of Allergy and Infectious Diseases, U.S.P.H.S., and by Dr. Henry C. Buswell and Bertha H. Buswell Research Fellowship. The authors express sincere appreciation for the cultures to Dr. George J. Hermann, Enteric Bacteriology Unit, Center for Disease Control, Atlanta, Georgia.