TABLE IV.
Effect of Several Inhibitors on *Cl. histolyticum* Collagenase.

Substance	mM	Inhibition, %
Mercuric chloride	.05	100
Phenylmercuric acetate	.05	87
p-Chloromercuribenzoate	.008	100
Copper sulfate	.01	82.5
Formaldehyde	.30	100

ganic mercurials, copper sulphate, and formaldehyde in relatively low concentrations (Table IV). More extensive studies on this phase of the problem are in progress.

Separation of a proteolytic enzyme activated by Fe^{++} and cysteine. By precipitation from *undialyzed* filtrates with 66% methanol at 0°C an enzyme preparation was obtained which was active on gelatin but not on collagen. This preparation was further purified by precipitation from 0.05 M acetate buffer at pH 4.6. The product was activated by Fe^{++} and cysteine and its properties more closely resembled the proteinase described by Kocholaty and Krejci(3) and van Heyningen (4).

Summary. A consistently reproducible method is described for producing *Clostridium histolyticum* filtrates with good collagenase activity and little or no toxin. The proteolytic enzyme which attacks collagen has been purified 400 times based on activity per unit of nitrogen. The "collagenase" activity is not enhanced by Fe^{++} and cysteine. The collagenase activity is inhibited by specific *Cl. histolyticum* antisera in low concentrations. This inhibition is not proportional to the antitoxic value of the antiserum. Normal rabbit, guinea pig, and horse serum in high concentrations partially inhibit collagenase activity. Collagenase is not inhibited by crystalline soy bean trypsin inhibitor. Collagenase activity is inhibited by reagents supposedly specific for amino groups and sulphydryl groups. It is also sensitive to heavy metals. Collagenase activity is destroyed at 50°C in dilute solutions of enzyme. A proteolytic enzyme, activated by Fe^{++} and cysteine, active on gelatin and not active on collagen is also present in *Cl. histolyticum* filtrates.

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Pteroylglutamic Acid-like Effect of Dehydroisoandrosterone on Growth of Certain Microorganisms.* (17971)

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During routine testing of a number of compounds for ability to reverse aminopterin inhibition of *Streptococcus faecalis* it was found that dehydroisoandrosterone acetate† was especially active. Further trials revealed that this steroid has pteroylglutamic acid (PGA) like activity for both *S. faecalis* and *Lactobacillus casei* and is capable of stimulating *Escherichia coli* in the presence of inhibiting levels of sulfanilamide. Several other steroids and 2 carcinogens were tested and a few found to have very slight PGA-like activity. Although the activity was small when compared with that of pteroylglutamic acid the ability of compounds such as sex hormones and carcinogens or their derivatives to stimulate cell division in a manner similar to that of pteroylglutamic acid could hardly fail to have great biological significance. Further experiments on steroids are under way. The data presented here are representative of a large number of tests made to determine the

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† We would like to express our appreciation to the Schering Corporation for furnishing us with the dehydroisoandrosterone acetate used in this experimental work.

TABLE I.
Pteroylglutamic Acid-like Effect of Dehydroisoandrosterone Acetate (DIA) on *Streptococcus faecalis* and on *Lactobacillus casei*.

Organism	Additions to medium, γ/10 ml	Photometric density 16 hr	Photometric density 40 hr	ml 0.1 N NaOH, 46 hr
S. faecalis	PGA			
	0	.02	.03	0.8
	.001	.17	.21	4.1
	.005	.29	.32	4.8
	DIA			
	20	.17	.29	5.1
	100	.24	.35	5.1
	400	.38	.55	5.3
L. casei	PGA			72 hr
	0			2.2
	.0004			5.8
	.001			7.5
	.005			8.9
	DIA			
	.25			2.9
	2.5			6.2
	10.			8.5
	300.			10.1

nature of the growth stimulating activity of dehydroisoandrosterone. It should be recalled that Barton-Wright *et al.*(1) found folic acid-like activity in chloroform extracts of liver several years ago.

Experimental. The organisms used for the present study were *Streptococcus faecalis* (American Type Culture Collection No. 8043), *Lactobacillus casei,* and a laboratory strain of *Escherichia coli.* The medium used for *S. faecalis* and *L. casei* was similar to that of Mitchell and Snell(2). For convenience the same medium with the omission of all vitamins except pantothenic acid was used for *Escherichia coli.* All organisms were incubated at 32°C for periods of time ranging from 16 to 72 hours. Titration of the lactic acid produced by *S. faecalis* or by *L. casei* was made at the times indicated in Table I. Turbidity measurements were made at intervals on a Coleman Junior spectrophotometer and recorded.

Results.‡ The ability of dehydroisoandrosterone acetate to stimulate the growth and acid production of *Streptococcus faecalis* and *Lactobacillus casei* is shown by typical results recorded in Table I. Results with PGA are also given for comparison. It may be seen that the steroid is required in amounts about 20,000 times as great as PGA to produce one-half maximum growth at 16 hours. However, this ratio differs with the incubation times and with the species of organism used. These data, as well as those in Table II, indicate that the activity of the steroid is not due to any possible contamination with PGA. Additional assurance that PGA contamination was not responsible for the activity was obtained by refluxing the steroid for 8 hours in 1 N HCl. This treatment did not materially affect the activity although PGA is almost completely destroyed by such a procedure. After 36 sub-cultures on dehydroisoandrosterone acetate alone *Streptococcus faecalis* still required PGA or dehydroisoandrosterone although the organism seemed to adjust itself to lower concentrations

1. Barton-Wright, E. C., Emery, W. B., and Robinson, F. A., *Biochem. J.,* 1945, v39, 334.

2. Mitchell, H. K., and Snell, E. E., *Univ. Texas Pub. No. 4137,* 1941, 36.

‡ At our request, Dr. E. L. R. Stokstad very kindly checked our results with dehydroisoandrosterone acetate obtained by him from the Schering Corporation. According to a personal communication he obtained activity similar to that reported here.

TABLE II.
Growth Stimulation of Various Organisms by Dehydroisoandrosterone Acetate (DIA) and PGA in the Presence of Inhibitors.

Organism and inhibitor	Additions to medium, γ/10 ml	Photometric density 16 hr	Photometric density 40 hr
Streptococcus faecalis	PGA		
Pteroylaspartic acid			
2 γ/10 ml	0	0	0
,,	.001	0	0
,,	.005*	0	0
	DIA		
,,	20	.07	.16
,,	50	.13	.26
,,	300	.41	.51
4 amino PGA	PGA		
.05 γ/10 ml	0	.08	.11
,,	.001	.11	.11
,,	.005	.15	.15
	DIA		
,,	20	.07	.12
,,	100	.11	.22
,,	300	.28	.48
Escherichia coli	PGA		
Sulfanilamide			
16 mg/10 ml	0	.04	.15
,,	.001	.08	.24
,,	.005	.09	.26
	DIA		
,,	20	.17	.26
,,	50	.11	.30
,,	400	.08	.22
	Thymine		
,,	500	.19	.24

* This quantity of PGA in the presence of inhibitor gave good growth (P.D. = 0.3) at 112 hours.

of dehydroisoandrosterone.

The mode of action of the sex hormone is not similar to that of thymine. This may be seen by examination of the data in Table II. The inhibition due to 4 amino pteroylglutamic acid or to pteroylaspartic acid appeared to be reversed competitively by dehydroisoandrosterone. Thymine reverses this type of inhibition non-competitively(3).

The action of the dehydroisoandrosterone in promoting growth of the test organisms closely simulates the activity of PGA but some differences seem to exist. At optimum concentration and with the medium used the steroid appeared to stimulate somewhat more growth with *Streptococcus faecalis* than was obtainable with PGA at any concentration. On the other hand the organisms tested did not reach maximum growth as quickly with dehydroisoandrosterone acetate as with PGA. It is not unlikely that the substitution for PGA occurs better for certain functions of the vitamin than for others. However, there is also a possibility that in the presence of the hormone the organisms are stimulated to produce PGA.

Summary. Dehydroisoandrosterone acetate was found to have pteroylglutamic acid-

3. Hutchings, B. L., Monat, J. H., Oleson, J. J., Stokstad, E. L. R., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRow, Y., *J. Biol. Chem.*, 1947, v170, 1323.

like activity for *Streptococcus faecalis, Lactobacillus casei* and sulfonamide-treated *Escherichia coli.* The activity of the steroid differs from that of thymine but is of the same order of magnitude.

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On Hemolysis Mediated by Non-Erythrocytic Antigens, Their Homologous Antibodies and Complement. (17972)

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Kondo(1) observed hemolysis in mixtures of sheep erythrocytes, suspensions of *Serratia marcescens,* and fresh guinea pig serum. He attributed lysis to the enhancement of bacterial hemolysin by guinea pig complement. A recent brief note by Fisher and Keogh(2) described the lysis by complement of erythrocytes which had adsorbed bacterial components and anti-bacterial antibodies. The observations of these authors have been extended in the present report.

Materials and methods. (1) *Antigens.* Trichloroacetic extracts(3) were prepared from *Salmonella typhosa* O 901 and from *Escherichia coli,* strain H. After dialysis against water, the extracts were concentrated to contain approximately 0.5 mg solids per ml, sodium chloride was added to obtain isotonicity, the reaction was adjusted to pH 7, and sterility was attained by Seitz filtration. In another experiment human plasma, in place of bacterial antigens, was used.

(2) *Antisera and complement.* Seitz filtered, heat-inactivated immune sera from four rabbits were employed. One of each of the immune sera was prepared by immunization with *E. coli, S. typhosa,* human serum, and washed sheep erythrocytes. For the detection of rabbit serum adsorbed on the surface of erythrocytes, a chicken immune serum against rabbit euglobulins, absorbed with rabbit serum albumin and pseudoglobulin, was used. Aliquots of immune sera, employed in some experiments, were freed of normal hemagglutinins by double absorption at 37°C (two 15 minute periods) with 0.5 ml packed erythrocytes for each ml of serum. The fresh pooled serum from at least 3 normal guinea pigs, diluted 10-fold in saline, served as complement.

(3) *Erythrocytes* and *"sensitization."* Erythrocytes from sheep blood preserved in Alsever's solution, rabbit cells from fresh defibrinated blood, and human type O cells from citrated blood were used. The cells were usually washed twice in saline, and a 1% suspension was then prepared, using as suspending fluid (a) antigen solution or immune serum dilution, respectively, and (b) salt solution (controls).* With occasional agitation, these cell suspensions were incubated one hour at 37°C, the cells were then washed twice in saline, and 1% cell suspensions in saline were prepared of both "sensitized" and "non-sensitized" (control) cells.

(4) *Hemolysis titration.* Twofold serial dilutions of antigen solution or of immune serum, respectively, in 0.2 ml amounts, were prepared in duplicate. To both series of tubes were then added 0.2 ml amounts of a 1:10 dilution of complement. One series of tubes received 0.2 ml amounts of 1% sensitized

1. Kondo, S., *Z. Immunitäts.,* 1923, v36, 76.

2. Fisher, S., and Keogh, E. V., *Nature,* 1950, v165, 248.

3. Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.,* 1933, v112, 76.

* Cells which were exposed to solutions of bacterial antigens or to serum proteins in the manner just described will be referred to as sensitized cells.