light upon the mechanism of its action but suggest that, as a therapeutic agent in women with hypo-pituitary-ovarian disorders, it should be very much more effective than the naturally-occurring estrogens. The clinical experience of one of us (G.V.S.) corroborates this.

Summary. Stilbestrol is about 100 times as active as estrone in causing the stimulation and release of adrenotropic and gonadotropic factors from the pituitaries of intact mature male rats. In castrated animals it is only about twice as active as estrone. Unlike estrone, the pituitary responses to stilbestrol are not influenced by the presence of the testes or the administration of progesterone, both of which depress the rate of inactivation of estrone as well as its pituitary stimulating activity. It would appear that the mechanism of stilbestrol's action upon the pituitary differs from that of the naturally-occurring estrogens.

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Antigenic Types of *Shigella alkalescens.*

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Until 1939, *Shigella alkalescens,* a member of the *Shigella* group, was considered to be an antigenically homogeneous species. In that year Assis[1] described as *S. alkalescens* type II a strain (No. 648) which differed both in biochemical characteristics and antigenic structure from the hitherto known strains comprising type I. In 1943, Stuart, Rustigian, Zimmerman, and Corrigan[2] demonstrated that type I strains contain 5 antigenic components, which they designated A, B, C, D and E. The major antigens A and B and the minor antigen C are present in all typical strains. The antigenic fractions D and E were found either singly or in combination. In May, 1944, Captain William H. Ewing, Sanitary Corps, United States Army, sent several strains, identified as *Shigella,* to the author to determine whether some of them may be identical with type II of *S. alkalescens.* Serological studies carried out in this laboratory revealed that Ewing's strain No. 2-193 differed from both types I and II of *S. alkalescens.* While these studies were in progress a *S. alkalescens* strain (No. 9731) was isolated from the feces of a healthy infant, which was not agglutinated to titer by antisera to types I and II as well as strain No. 2-193. The experiments reported in this communication reveal that strains No. 9731 and No. 2-193 represent hitherto undescribed types of *S. alkalescens.*

Material and Methods. The following strains were employed: (1) Strain No. 1-2, *S. alkalescens* type I, received from Captain Ewing; (2) Strain No. 648, *S. alkalescens* type II, obtained through the courtesy of Dr. Arlindo de Assis; (3) No. 2-193 sent by Captain Ewing; (4) No. 9731 isolated at this laboratory from the feces of a healthy infant.

Antisera were prepared in rabbits. Two rabbits were injected intravenously with 3 ml of each bacterial suspension. Two injections, one week apart, were given. Ten days after the last injection the animals were bled and the serum was collected.

In addition to these sera, 2 anti-*S. alkalescens* type I sera were employed: (1) No. 842 prepared at this laboratory and (2) a serum obtained through the courtesy of Dr. E. G. E. Murray, McGill University, Montreal.

Sera used in the slide agglutination test for the determination of the various types belonging to the genus *Shigella* were procured

[1] Assis, A., *O Hospital,* 1939, **15**, 447 and 655.

[2] Stuart, C. A., Rustigian, R., Zimmerman, A., and Corrigan, F. V., *J. Immunol.,* 1943, **47**, 425.

TABLE I.
Agglutinin Titer of Different *S. alkalescens* Sera.

S. alkalescens sera	Strains of *S. alkalescens*			
	Type 1 No. 1-2	No. 2-193	No. 9731	Assis type II
Type I (No. 1-2)	*1:1600*	1:10	1:100	1:10
" I (No. 842)	*1:1000*	<1:10	<1:10	<1:10
" I (McGill)	1:1000	<1:10	1:100	<1:10
No. 2-193 (a)	1:10	*1:2000*	1:10	1:80
(b)	<1:10	*1:4000*	1:20	1:160
No. 9731 (a)	<1:10	<1:10	*1:1000*	<1:10
(b)	1:10	<1:10	*1:8000*	1:20
Assis type II	1:80	1:320	1:320	*1:4000*

through the kindness of Dr. A. J. Weil, Lederle Laboratories, Pearl River, New York.

Unless otherwise indicated, all agglutination and agglutinin-absorption tests were carried out in test tubes 100 mm in length and 13 mm in width. Serial dilutions of the respective sera (1:10, 1:20, etc.; 1:100, 1:200, etc.; 1:1000, 1:2000, etc.) were prepared, using different pipettes for every dilution. As antigens, bacteria suspended in 0.4% formaldehyde-saline solution were used. The resulting agglutination was read with the naked eye after various periods of incubation at 55°C.

Results. The 4 strains under investigation (No. 1-2, No. 2-193, No. 9731, and Assis type II) were studied with respect to morphology, motility, and biochemical characteristics. The organisms are gram-negative bacilli. They grow profusely in infusion broth as well as on Endo agar. They are non-motile. The colonies on plain agar are smooth. With the exception of Assis type II these strains were found to be identical in biochemical activities. They produced acid without gas from glucose, maltose, mannitol, rhamnos, xylose, dulcitol, and sorbitol. They failed to form acid from lactose, sucrose, and salicin. Gelatin was not liquefied. Indole was formed. In litmus milk they first produced acid and later alkaline reaction. Strain No. 648, type II of Assis, differed from the other strains in its inability to produce acid from rhamnose and dulcitol and its capacity to form acid from salicin. In view of these differences in biochemical activity the question arises as to whether or not this strain will ultimately be classified as *S. alkalescens;* rather, it may belong to *S. paradysenteriæ.* Thus, biochemically, the hitherto undescribed strains No. 2-193 and No. 9731 present the pattern of *S. alkalescens* type I.

The antigenic structure of these strains was investigated by means of agglutination tests. The agglutinin titer of sera against the 4 strains was determined. The experiments were carried out in triplicate. Final readings were made after incubation at 55°C for 18 hours. The results are presented in Table I.

It may be seen from this table that all sera tested agglutinated the homologous strains in titer ranging from 1:1000 to 1:8000. It is also evident that strains No. 2-193, No. 9731 and Assis type II were agglutinated only to a fraction of the titers of three Type I antisera. This indicates that strains No. 2-193 and No. 9731 differ in their major antigens from *S. alkalescens* types I and II. Furthermore, it is clear that the hitherto undescribed strains No. 2-193 and No. 9731 are antigenically unlike.

Absorption experiments were carried out to determine whether agglutination of heterologous organisms by antisera to Assis type II and No. 2-193 is due to antigenic components common to these strains. Anti-Assis type II serum and anti-No. 2-193 serum in dilutions of 1:20 were absorbed with a suspension of strain No. 2-193 in one experiment and a suspension of Assis type II in another. The suspensions were obtained by harvesting the growth on plain agar (Kolle flasks) in 0.4% formaldehyde-saline solution. After centrifu-

TABLE II.
Agglutinin Titer of Native and Absorbed *S. alkalescens* Sera.

S. alkalescens strains	Anti-Assis type II serum			Anti-No. 2-193 serum		
	Native	Abs. with No. 2-193	Abs. with Assis type II	Native	Abs. with No. 2-193	Abs. with Assis type II
Assis type II	*1:4000*	*1:4000*	<1:40	1:200	<1:40	<1:40
No. 2-193	1:400	<1:40	<1:40	*1:4000*	<1:40	*1:4000*
No. 9731	1:40	1:40	1:40	<1:40	1:40	1:40
No. 1-2 (type I)	1:40	1:40	1:40	<1:40	<1:40	<1:40

gation the sera were added to the sediments. The tubes were shaken thoroughly and incubated at 55°C for 4 hours. Unabsorbed serum in like dilution was treated in the same manner. The tubes were centrifuged and absorption was repeated. Then the supernates as well as the native sera were tested for the presence of agglutinins against the 4 strains under investigation. The experiment was carried out in duplicate. The results are summarized in Table II.

The conclusion may be drawn that the hitherto undescribed strain No. 9731 shares no demonstrable antigenic components with Assis type II and strain No. 2-193. On the other hand, strain No. 2-193 contains a small antigenic fraction in common with Assis type II; its major antigenic components, however, differ from the major antigens of Assis type II. Thus, the 4 strains represent 4 different antigenic types.

The conclusion that strains No. 2-193 and No. 9731 as well as Assis type II differ in antigenic structure from *S. alkalescens* type I is further substantiated by the following observation. Fifteen strains of *S. alkalescens* type I, available at this laboratory, were tested with antisera to *S. alkalescens* types I and II as well as strains No. 2-193 and No. 9731. It was observed that all 15 strains were strongly agglutinated to high titer by anti-type I serum, but not at all or only to a small fraction of the titers of the antisera against Assis type II, No. 2-193, and No. 9731.

It is well known that *S. alkalescens* type I may be agglutinated by certain anti-*S. paradysenteriæ* sera. It seemed of interest to determine, therefore, what relationship, if any, strains No. 9731 and No. 2-193 have to paradysentery bacilli. To this end the following experiment was carried out. Various anti-*Shigella* sera, obtained from Lederle Laboratories through the courtesy of Dr. A. J. Weil, were used. They included sera against the following species and types: (1) *S. dysenteriæ* (Shiga), (2) *S. sonnei*, (3) *S. ambigua* (Schmitz), (4) *S. alkalescens*, (5) *S. paradysenteriæ* (polyvalent), and (6 to 19) Flexner types I to XIV representing V, W, X, Y, Z of Andrews and Inman as well as Boyd's types 103, P119, 88 (Newcastle), 170, P288, D1, D19, P143 and 274. One drop of these diagnostic reagents was mixed on a slide with one drop of the bacterial suspension previously heated at 100°C for 30 minutes. Agglutination was read with the naked eye. The following results were obtained: *S. alkalescens* type I (No. 1-2) was agglutinated only by anti-*S. alkalescens* serum. The strains No. 9731 and Assis type II showed a trace of clumping with serum against Flexner type XIII, but none with any of the other sera. Strain No. 2-193 was strongly agglutinated by anti-type XIII serum and not at all by the others. Dr. A. J. Weil also observed agglutination of this strain by anti-type XIII serum. In this connection mention should be made of the observation of Boyd[3] to the effect that *S. alkalescens* and Flexner type P274 cross-agglutinate, although they fail to remove the heterologous agglutinins on absorption. Further studies are necessary to determine the antigenic relationship of *S. alkalescens* No. 2-193 and Flexner type XIII.

The antigenic relationship of the hitherto undescribed types of *S. alkalescens* to the typhoid-paratyphoid group was also studied. Suspensions of *E. typhosa*, *S. paratyphi*, *S. enteritidis*, and *S. typhi murium* were tested with the 4 different anti-*S. alkalescens* sera.

[3] Boyd, J. S. K., *J. Hygiene*, 1938, **38**, 477.

It was found that neither of the antisera against types I and II and strains No. 9731 and No. 2-193 agglutinated these organisms to more than a very small fraction (1% or less) of the titers. Likewise, the suspensions of the *S. alkalescens* strains were not agglutinated or agglutinated only to a fraction of the titers of antisera against the typhoid-paratyphoid organisms. It is evident, therefore, that the 4 types of *S. alkalescens* do not share any major antigens with the typhoid-paratyphoid organisms studied.

Summary and Conclusions. Two hitherto undescribed antigenic types of *S. alkalescens* have been reported. The representative strains have the morphological, cultural, and biochemical characteristics of *S. alkalescens* type I and do not share major antigenic components either with each other or with types I and II. One of the strains was isolated by Captain Ewing, the other at this laboratory. At the present time, therefore, *S. alkalescens* comprises 3 antigenic types, namely, type I composed of 4 sub-types, and the 2 new types described here. It is doubtful whether type II of Assis will ultimately be classified as *S. alkalescens.*

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Effect of Lowered Blood Supply and of Glucose-1-Phosphate on Healing of Bone Fractures.*

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Promotion of bone fracture healing is a serious problem at all times in the advanced age group of the population, because, with advancing age, there is an increased susceptibility to fractures, and difficulty and delay in their healing is a fairly common occurrence. The study of therapeutic measures to promote fracture healing in older individuals has been retarded up to now by lack of a method of producing a delay in the union of fractures comparable to that found in old age.

In this communication there is described a simple experimental procedure which yields a long delay in the healing of bone fractures. The technic consists simply of ligaturing the femoral artery of a limb and thereby reducing the blood supply to the affected bone. By this technic it is hoped that therapeutic measures can be tested which eventually will prove to be of benefit in the treatment of delayed union in the aged. The results obtained on rats by this technic will be presented below.

The high alkaline phosphatase activity of calcifying bone has attracted the attention of investigators since the pioneer work of Robison. Robison's theory[1] that the phosphatase acted to raise the product of calcium and phosphate ions by liberating inorganic phosphate from organo-phosphates in the blood faced the difficulty that the acid-soluble organic ester content of the blood plasma is small. It has been observed that glycogen increases in hypertrophic cartilage prior to ossification and disappears during ossification (Harris,[2] Glock[3]). The presence of the en-

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1 Robison, R., *The Significance of Phosphate Esters in Metabolism,* 1932, New York Univ. Press, N.Y.

2 Harris, H. A., *Nature,* 1932, **130**, 996.

3 Glock, G. E., *J. Physiol.,* 1940, **98**, 1.