stan-3-one.

Summary and conclusion. Incubation of testosterone with an homogenate of liver from female rats led to formation of 17β-hydroxyandrostan-3-one. This steroid has been postulated as a possible intermediate in the metabolism of testosterone but up to now has been neither isolated from natural sources nor detected in metabolism experiments *in vivo* or *in vitro*.

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Clinical, Bacteriological, and Serological Observations of two Human Volunteers Following Ingestion of *Escherichia coli* O127:B8. (22338)

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An epidemic of infantile diarrhea due to *Escherichia coli* O127:B8 reported by Stulberg and co-workers(1) has been further studied serologically by Stulberg, Zuelzer and Page(2). In an effort to produce specific antibodies in humans to be used as positive control sera for the serological portion of their studies, 2 adult volunteers ingested O127:B8 organisms following the methods employed by Ferguson and June(3) and June, Ferguson and Worfel(4) in their classical feeding experiments using *E. coli* O111:B4 and O55:B5. Since volunteer studies using O127:B8 have not been published, and because clinical, bacteriological and serological results of seeming interest were obtained, this experience is being reported.

Methods. Two adults, white males, age 28 and 32, participated in this study. Each man was in general good health and had been free of diarrhea of any nature for at least one year prior to this experiment. *Inocula*: *E. coli* O127:B8 cultures obtained from 3 infants during their diarrheal disease(1) were inoculated separately on brain heart infusion agar slants. After 18 hours incubation at 37°C, the growth from each slant was harvested in saline. The 3 suspensions were pooled and washed once in saline. The pooled suspension was diluted in saline and colony counts estimated the inocula for the two volunteers to contain 4,000,000 and 8,000,000 organisms respectively. In each instance the inoculum was contained in 1 ml. Each inoculum was added to one-half pint of pasteurized milk which was kept on ice until ingested, approximately one hour later. A challenge inoculum of 8,000,000 organisms prepared in a similar manner was ingested by volunteer A 32 days following the initial ingestion. One pre-ingestion blood sample and 3 pre-ingestion rectal swabs were obtained from each volunteer. Following ingestion, rectal swabs and venous blood samples were collected daily for about 2 weeks and later at approximately 3 day intervals. A detailed record of symptoms was kept by each volunteer. *Bacteriological and serological examinations*: Rectal swabs were inoculated immediately on blood agar and MacConkey's bile salts agar plates. After 20 to 24 hours, colonies appearing on the blood plates were tested for agglutinability with OB rabbit antiserum prepared with *E. coli* O127:B8, strain 9782 obtained from Dr. William Ferguson. Standard bacterial agglutination tests using OB and O antigens prepared with *E. coli* O127:B8 culture No. 2220 were performed according to the method described by Kauffmann(5). Saline suspensions of the organisms grown for 18 hours on brain heart infusion agar were used as antigens. Hemagglutination tests were performed

by a technic developed by Stulberg, *et al.*(2), based on the methods of Neter, *et al.*(6), and Wheeler and Taylor(7).

Results. Symptoms: Both volunteers experienced a sharply defined illness beginning with an explosive liquid stool. Liquid diarrhea, abdominal cramps, nausea and mild to moderate malaise were the predominating symptoms. In volunteer A the incubation period was 17 hours, the illness lasted 32 hours and with 4 diarrheal stools and an afebrile course was classified as mild. In volunteer B the incubation period was 7 hours, the duration 46 hours and with 10 diarrheal stools and a per oral temperature elevation of 100°F, the illness was classified as of moderate severity. No symptoms were recorded following the challenge ingestion by volunteer A.

The presence of *E. coli* O127:B8 in fecal cultures was determined by slide agglutination tests with 5 to 15 colonies from each blood plate. Organisms of this serotype were recovered from both volunteers 24 hours after ingestion and for a period of 5 days in one volunteer and 12 days in the other. In volunteer A, *E. coli* O127:B8 was found in the stools in increasing amounts and on the sixth day after ingestion, was the only organism present. The stool flora then gradually reverted to its pre-ingestion status. From the 13th through the 26th day 5 negative cultures were obtained. On the 32nd day a pre-challenge ingestion culture produced scanty growth of O127:B8. One day following the challenge ingestion moderate growth was present. Additional post-challenge cultures were not obtained.

Serological examination: Results of the standard agglutination and hemagglutination tests are shown in Table I. Serum hemagglutinins were detected as early as 3 days after ingestion and bacterial agglutinins with boiled cultures within 5 days. Titers with both tests continued to rise until the eighth or ninth day and remained near the peak level for the remainder of the initial experiment. Bacterial agglutinations with living cultures were, in every instance, negative at a serum dilution of 1:10. In volunteer A on the 32nd post-ingestion day bacterial agglutination was negative at a serum dilution of 1:10 while the hemagglutinin titer had dropped to 1:40. Three days following the challenge ingestion bacterial agglutinin and hemagglutinin titers were 1:40 and 1:160 respectively.

TABLE I. Results of Serological Examinations.

Days following ingestion	Agglutination titers* at different intervals					
	Volunteer A			Volunteer B		
	Bacterial		Hemagglutination	Bacterial		Hemagglutination
	O	OB		O	OB	
0	0†	0	20	0	0	10
1	0	0	20	0	0	10
2	0	0	40	0	0	20
3	0	0	80	0	0	40
4	0	0	80	0	0	40
5	10	0	80	10	0	40
6	20	0	320	20	0	80
7	160	0	320	40	0	80
8	80	0	320	40	0	160
9	160	0	640	40	0	160
12	80	0	320	40	0	80
15	80	0	320	20	0	80
18	80	0	320	20	0	80
32	0	0	40	nt		nt
3 (post-challenge)	40	0	160	nt		nt

* Titers are shown as reciprocal of highest dilution in which definite agglutination was observed.
† Zero indicates negative result at dilution of 1:10.
nt = not tested.

Discussion. Our results are interpreted as evidence of a definite bacterial, clinical and serological response by 2 adult human volunteers to the ingestion of *E. coli* O127:B8 organisms. The clinical responses, including the incubation periods and the duration of symptoms, are similar to those observed by Ferguson with serotypes O111:B4 and O55:B5. It is interesting to note, however, that volunteer B suffered an illness of moderate severity after the ingestion of only 4,000,000 organisms. In no instance did Ferguson record an illness of more than slight severity with dosages of less than 530,500,000 organisms with O111:B4 or 143,000,000 organisms with O55:B5. The question of possibly greater pathogenicity for adults of serotype O127:B8 in comparison with O111:B4 and O55:B5 is raised by these observations.

Serological tests showed comparable rises in antibody titer for the 2 volunteers, the hemagglutinin titers being consistently higher than the bacterial agglutinin titers. This is

in agreement with the results obtained by Neter(8) in a comparison of the two methods, using sera obtained from volunteers in the feeding studies of Ferguson. The antibody response detected by bacterial agglutination when using boiled cultures as antigen could not be demonstrated when living cultures were used. This is in accord with Ferguson's results.

In both volunteers *E. coli* O127:B8 appeared in the stool 24 hours after ingestion of the cultures and increased in numbers during the following few days. The persistence of the organisms in substantial numbers for several days after the complete subsidence of symptoms is interesting and is in accord with the findings of Ferguson and co-workers with the O111:B4 and O55:B5 serotypes.

The results of the challenge feeding suggest that protection from clinical symptoms may have been afforded by the initial ingestion. It is interesting that the pre-challenge culture was positive although 5 previous cultures had been negative. Properly designed human experiments seem indicated to study a possible post-ingestion immune response and to delineate the duration and consistency of fecal elimination of the organism during convalescence.

Summary. 1. Two healthy, adult white, male volunteers ingested, respectively, 4 and 8 million organisms of *E. coli* serotype O127:B8. 2. Symptoms of mild to moderate gastroenteritis appeared in both subjects 7 to 17 hours after ingestion of the organisms and persisted for 32 to 46 hours. 3. By daily rectal swab cultures, *E. coli* O127:B8 organisms were recovered within 24 hours after ingestion and were found during 5 days in one and 12 days in the second volunteer. 4. Hemagglutinins were detected 3 days and bacterial agglutinins 5 days following ingestion. Antibody titers in both subjects rose rapidly, reaching peaks by the eighth to ninth day. Hemagglutination titers were consistently higher than bacterial agglutination titers. 5. Challenge ingestion of 8,000,000 organisms by one volunteer was followed by bacterial and serological response without appearance of clinical symptoms.

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