acid. Thus, the factors involved in the induction of type transformation of Flexner bacilli require further study both concerning the parent organisms and the properties of filtrates.

The demonstration of induced type transformation in *Shigellae* is not only of theoretical interest,[5] but it also cannot fail to influence our viewpoints on problems of the biology of the enteric flora. These have just been discussed at some length,[7] and it may suffice here to recall this aspect of induced type transformation.

Summary. Induced type transformation of *Sh. paradysenteriae* (Flexner) has been obtained in 3 out of 225 experiments. The inducing principle is contained in filtrates of broth cultures.

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Ovarian Hyperemia in the Immature Rat as a Pregnancy Test in Equids.

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Early diagnosis of pregnancy in equids by means of a biological reaction was studied by Cole and Erway.[1] A new method has been introduced by Zondek and Sulman[2] based on the ovarian hyperemia in immature rats injected with serum gonadotrophin. The same reaction proposed by Salmon *et al.*,[3] as a 6-hour test for human pregnancy, was challenged by Farris[4] and accepted with restrictions by Bunde.[5] Recently Zondek and Sulman[6] have recommended it again as a 24-hr test for the diagnosis of equine pregnancy. This note deals with the evaluation of the new test which was employed as a preliminary step for the preparation of mare gonadotrophin.

Experimental. Blood samples from 38 mares and 4 female donkeys obtained between 42 and 138 days after natural insemination were tested. Two infantile female rats weighing 40-55 g were injected subcutaneously with 1 and 2 cc respectively of each serum and killed 24 hours later. The ovaries were inspected in daylight and the response considered positive when they were enlarged and their color matched that of the kidney or the spleen. Pale yellow and small sized ovaries were indications of a negative response. Occurrence of pregnancy was assumed when one or both of the injected rats showed a positive response. With this technique and using rats from the same colony, positive reaction was always obtained 24 hours after the subcutaneous injection of 10 I.U. of a standard preparation of equine gonadotrophin. Therefore, when a positive response with 2 cc of serum was obtained, a blood concentration of at least 2,500 I.U. of gonadotrophin per liter was assumed to be present. Of course this is not an exact quantitative measurement but only a routine approximation.

A careful record was kept of each animal until evidence of pregnancy was established either by obvious abdominal enlargement or by subsequent parturition.

Results. Table I gives the results of the test with the serum of 38 mares and 4 female donkeys and the number of days between the natural insemination and the taking of

* Fellow of the J. S. Guggenheim Memorial Foundation.

1 Cole, H. H., and Erway, J., *Endocrinology*, 1941, **29**, 514.

2 Zondek, B., and Sulman, F., *Nature*, 1945, **3932**, 302.

3 Salmon, U. J., *et al.*, *J. Clin. Endocrinology*, 1942, **2**, 167.

4 Farris, E. J., *Am. J. Obst. and Gyn.*, 1944, **48**, 200.

5 Bunde, C. A., *Am. J. Obst. and Gyn.*, 1947, **53**, 317.

6 Zondek, B., and Sulman, F., *Endocrinology*, 1947, **40**, 322.

TABLE I.
Individual Data and Results of the Pregnancy Test in Mares and Female Donkeys.

Case No.	Age (years)	Wt (kg)	Days after insemination	Hyperemia reaction	Remarks
A. Mares					
1	9	501	90	Positive	Parturition, 330 days pregnant
2	13	470	78	"	Not pregnant*
3	12	400	80	"	Parturition, 327 days pregnant
4	15	336	72	"	" 328 " "
5	13	415	88	"	" 341 " "
6	18	343	68	"	Not pregnant*
7			72	"	Pregnant
8			56	Negative	Not pregnant
9			42	"	" "
10			78	Positive	Parturition, 327 days pregnant
11	15	370	80	"	Pregnant
12	9	370	71	"	"
13	13	420	81	Negative	Not pregnant
14	6	430	83	"	" "
15	10	450	69	Positive	Pregnant
16		400	62	Negative	Not pregnant
17	16	400	62	"	" "
18	5	330	76	Positive	Pregnant
19	11	390	78	"	"
20			87	"	"
21	12	410	85	Negative	" †
22	16	460	76	"	Not pregnant
23	9	340	64	Positive	Pregnant
24			114	Negative	Not pregnant
25			138	Positive	Parturition, 350 days pregnant
26			138	"	" 310 " "
27			138	"	" 335 " "
28	11	473	74	"	Not pregnant*
29	4	300	71	"	Pregnant
30	6	450	69	"	"
31	7	490	75	Negative	" †
32	5	255	57	"	Not pregnant
33	6	345	70	Positive	Pregnant
34	5	305	73	"	"
35	6	408	63	Negative	" †
36	13		65	"	" †
37	9	390	71	"	Not pregnant
38	7	370	61	Positive	Pregnant
B. Female donkeys					
1			75	Negative	Not pregnant
2			128	"	" "
3			133	Positive	Parturition, 368 days pregnant
4			113	"	" 357 " "

* False-positive tests.
† False-negative tests.

the blood sample. The clinical condition of the animal agreed with the biological test (both positive and negative reactions) in 35 cases. It did not agree in 7 instances. Four mares definitely pregnant gave negative test 85, 75, 65 and 63 days after fertilization. On the other hand, 3 non-pregnant mares gave positive test 78, 74 and 68 days after insemination.

Bunde[5] pointed out that in human pregnancy, by employing the same hyperemia test, there occurred only false-negative test, *i.e.* negative reaction with urine of pregnant women. In equids, however, there occurred both types, namely negative test with serum of pregnant mares and positive test with serum of non-pregnant ones. The false-negative tests are difficult to explain for, according to the data of Day and Rowlands,[7] the high circulating level of gonadotrophin in mares occurs between the second and the fourth month of

[7] Day, F. T., and Rowlands, I. W., *J. Endocrinology*, 1940, **2**, 255.

pregnancy. It would be necessary to assume that in the 4 false-negative cases cited in Table I, the concentration of blood gonadotrophin, when the serum was tested, was less than 2,500 I.U. per liter, despite the fact that it was 63 to 85 days after definitely established fertilization. The false-positive tests, *i.e.* positive reaction with serum of non-pregnant mares, occurred in 11-, 13- and 18-year-old animals. It is possible that age may increase the blood level of gonadotrophin in equids more markedly than in postmenopausal women, but it is unknown to what extent.

Since positive or negative responses are unreliable in 17% of the cases, it seems that the ovarian hyperemia test in immature rats can be used only as a first approach to the exact diagnosis of pregnancy in equids.

Summary. The ovarian hyperemia reaction in immature rats was studied following the subcutaneous injection of equine serum from 38 mares and 4 female donkeys, 42-138 days after natural insemination. The accuracy of this 24-hour pregnancy test was 83%, failures being observed both by negative reaction in 4 pregnant cases and positive reaction in 3 non-pregnant ones.

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Effect of Ozonized Oxygen on Anti-A, Anti-B, and Anti-Rh Typing Sera.*

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Reports of attempts to modify agglutinating and "blocking" antibodies against human erythrocytes have recently appeared in the literature. Boyd,[1] following the lead of Tyler,[2] reported on the effect of photo-oxidation on isohemagglutinins, anti-A and anti-Rh in the presence of Eosin Y as a sensitizer. Photo-oxidation produced by photoflood lights gradually destroyed the agglutinating activity but did not convert any of the agglutinins studied into "inhibiting," "incomplete," or "blocking" antibodies. An exposure of 15½ hours to these photoflood lights was sufficient to destroy completely the anti-Rh agglutinin. Anti-A agglutinin, however, required an exposure of 65 to 71 hours to destroy all agglutinating activity. Although it seems likely that the action of the photoflood lights in Boyd's experiments was that of oxidation, the possibility remains, nevertheless, that some other photo-dynamic effect may have played a part in modifying the agglutinins. It seemed desirable to us, therefore, to determine the effects on such agglutinins using a different oxidizing agent.

Materials and Methods. In this study ozone was employed to determine the effect of oxidation on isohemagglutinating antibodies, since this agent has useful attributes not possessed by other oxidizing agents. There are, for example, no added end products of the reactions other than those formed from the use of O_3. Ozone is a powerful oxidizing agent that will combine with unsaturated carbon atoms and form ozonides. The normal mode of action of ozone is solely an addition with no secondary oxidation. Furthermore, the hydrogen atoms attached to unsaturated centers are left undisturbed.

Two cc quantities of the selected sera were subjected to ozonized oxygen having a concentration of approximately one part ozone to 1000 parts of oxygen. Volume concentrations were ascertained by the iodine liberation technique in which free I is liberated from

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1 Boyd, William C., *J. Exp. Med.*, 1946, **83**, 221.

2 Tyler, A., *J. Immunol.*, 1945, **51**, 157.