

II). Of interest are the experiments on Rabbit 3 in Table III. On October 20 an area over the abdomen was shaved. Seven days later the application of 50 units of insulin to this area failed to decrease the blood sugar (in fact, there was a temporary rise in the blood sugar probably due to the excitement manifested by this animal). On the following day, this same abdominal area, upon which the previous application of insulin failed to produce any effect, was reshaved and 50 units of insulin rubbed in approximately 15 minutes later. There was a marked fall in the blood sugar requiring the intravenous administration of dextrose to overcome the hypoglycemic convulsions.

*Conclusions.* The absorption of insulin by the skin of rabbits is dependent upon the integrity of the integument. The intact skin shows little or no absorption, whereas a recently abraded skin, such as produced by shaving, permits the absorption of an appreciable amount of insulin.

We are indebted to the Eli Lilly Company for a generous supply of powdered insulin.

### 9007 C

#### Fluctuations in Type 2 Pneumococcus Antibody During the Menstrual Cycle.\*

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In the course of measuring the duration of the protective power of the sera of a number of persons who had been fed pneumococcal vaccine, fluctuating values for Type 2 were observed in the blood of 2 women. In the case of A.E.T., No. 10,† 1 cc. serum from blood drawn at 2 different times early in the investigation protected mice against approximately 500 fatal doses. Seven and a half months later the serum protected against only minimal numbers of pneumococci (*ca.* 5 fatal doses). However, after a lapse of 27½ months more the blood again protected well (*ca.* 5000 fatal doses per cc. See Table, 1/28/35). The other person's (L.P., No. 18†) blood possessed no protective power when first examined, then acquired

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† The initials and the numbers are those used in the report, "Oral Immunization of Humans against the Pneumococcus." *J. Immunol.*, 1934, **27**, 307.

a small amount (*ca.* 50 fatal doses per cc.) which was lost after 12 months. Thirty-three months after the first determination the blood contained a considerable amount of antibody (*ca.* 5000 fatal doses per cc.). The fact that this sample of blood had been collected one day before menses began (January 14. See Table) suggested that the observed rise and fall in protective antibody for Type 2 pneumococcus might be related to the menstrual cycle. Inquiry revealed that the most recent sample of blood from A.E.T. which protected had been taken on the 8th day of the cycle. Two months later the first of 4 additional tests, done at different stages of the menstrual cycle over a period of 2 months, was carried out. Fluctuating concentrations were found in the serum of both individuals.

Others have reported that the bactericidal power of blood for a variety of organisms is related to the stage of the menstrual cycle. Geller and Sommer<sup>1</sup> reported a drop in bactericidal power of whole blood for hemolytic streptococci just before and during the first part of the menses followed by a rise during the latter part, or soon after. Dresel and Keller<sup>2</sup> reported that serum was bactericidal for anthrax bacilli during menstruation but not at other times. These authors considered this property to be non-specific in the immunological sense. Molinengo<sup>3</sup> found that the destructive power of blood for several bactericidal species derived from the vagina sinks in the premenstrual period and rises immediately following the end of menses.

Jungeblut and Engle<sup>4</sup> found that one individual's serum neutralized poliomyelitic virus on the first day of menses, but failed to do so on the 15th day of the menstrual cycle, and that another person's serum neutralized on the 15th day but not on the first day. They suggest that the power to inactivate the virus may change in accordance with fluctuations in endocrinal balance. Aycock<sup>5</sup> has recently reported that 4 out of 5 castrated female monkeys developed poliomyelitis following intranasal instillation of the virus while only one out of 5 similarly treated animals which received injections of estrin prior to the viral instillation succumbed. Söda,<sup>6</sup> however, reported no difference in the formation of normal agglutinins and bacteriolysins, nor immune agglutinins and bacteriolysins for typhoid bacilli in control and ovariectomized rabbits.

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<sup>1</sup> Geller, F. C., and Sommer, W., *Arch. f. Gynak.*, 1927, **31**, 293.

<sup>2</sup> Dresel, E. G., and Keller, W., *Z. Hyg.*, 1922, **97**, 151.

<sup>3</sup> Molinengo, L., *Gior. di Batt. e Imm.*, 1934, **13**, 379.

<sup>4</sup> Jungeblut, C. W., and Engle, E. T., *J. Immunol.*, 1933, **24**, 267.

<sup>5</sup> Aycock, W. Lloyd, *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 573.

<sup>6</sup> Söda, G., *Tarivan Igakkai Zasshi*, No. 269, August, 1927. (Summary in German.)

TABLE I.  
Protective Antibody Against Type 2 Pneumococcus at Different Stages of the Menstrual Cycle.

Dose cc.	Subject L. P., No. 18												Subject A. E. T., No. 10											
	1 day before menses		12th day of cycle		2d day of menses		20th day of cycle		9th day of cycle		8th day of cycle		14th day of cycle		1st day of menses		21st day of cycle		9th day of cycle					
	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.	Con.	Ser.				
10-9	2.3	S.S.	S.1	S.1	S.2	S.S.	S.1	S.S.	S.S.	S.S.	S.S.	S.1	S.1	S.2	S.S.	S.1	S.S.	S.S.	S.S.	S.S.				
10-8	1.1	S.S.	1.1	2.2	1.1	S.S.	1.1	S.S.	1.1	S.S.	1.1	S.S.	1.1	S.S.	2.1	S.S.	2.1	S.S.	1.1	S.S.				
10-7	1.1	S.S.	2.2	S.1	1.1	S.S.	1.1	S.S.	1.1	S.S.	1.1	S.S.	2.2	S.1	1.1	S.S.	2.1	S.S.	2.1	1.1	S.S.			
10-6	1.1	S.2	1.1	2.3	2.1	S.3	1.1	3.3	1.2	S.1	1.1	S.S.	1.1	2.1	2.1	S.S.	1.1	S.S.	2.1	1.2	2.2			
10-5				1.1	2.2	2.2	2.1	2.1	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	1.1			

All intervals in relation to the menstrual cycle were determined by observation except that for A.E.T. on 1/28/35 which was calculated on 3/29/35. June 8, 1935, was the 9th day by observation (A.E.T.); menses started about 7 days sooner than expected. S. = Mouse survived. The numerals give the number of days between the injection and time of death of the mouse. Two mice were injected with each dose of culture. Con. = Control mice. Ser. = Serum treated mice.

In the present experiments, the protective antibody was measured by injecting mice intraabdominally with a mixture of 0.20 cc. undiluted serum and 0.20 cc. pneumococcal culture diluted to contain the desired dose. The same strain of pneumococcus was used throughout all the experiments recorded here as well as those previously reported.

Blood was collected from L.P. on the 2d, 9th, 12th, and 20th days of the menstrual cycle (not all in the same cycle). (See table and chart). The protective antibody content is high on the second day. It is lower on the 9th day. On the 12th day the serum possesses no protective power. On the 20th day the titre is moderate once more. A similar fluctuation in protective power can be seen to have occurred in the case of A.E.T. The titre on the first day is high. In the 9th-day sample the concentration is considerably lower, and in that of the 14th day there was little or no Type 2 antibody. In the 21st day sample the concentration was about the same as in the blood drawn on the 9th day.

Protective Antibody Against Type 2 Pneumococcus at Different Stages of the Menstrual Cycle.

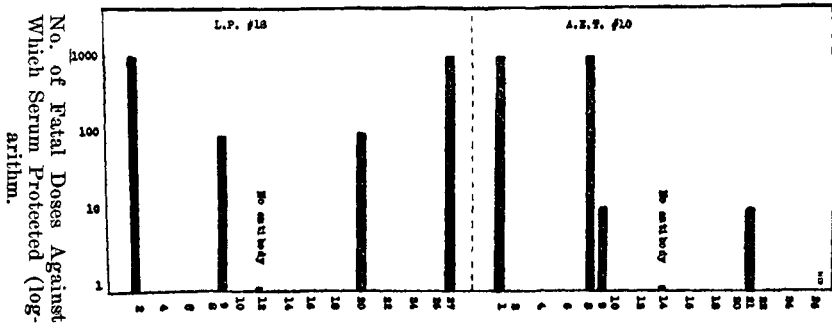


FIG. 1.  
Day of Menstrual Cycle.  
The vertical bar above the 27th day (L.P.) represents the value for the "day before" shown in the table.

There were fluctuations also in the Type 1 antibody content, but these were less marked than in the case of Type 2. The highest values for L.P.'s serum were obtained on the "day before", on the 2nd day and on the 9th day, that is on the same days when Type 2 values were high. In the case of A.E.T. the highest values for Type 1 were observed on the 1st and 8th days and lower values on the 9th, 14th and 21st days.

This rise and fall in titre suggests a relationship between antibody content and endocrinal factor, at least in some women. It will be observed that the protective power was high in the sera of both

subjects during menstruation and for several days after, and then fell off. It appears to rise again before the onset of the next period. Frank and Salmon<sup>7</sup> have recently reported a maximal concentration of gonadotropic hormone in the blood of normal, menstruating women between the 9th and 12th days. Frank and Goldberger<sup>8</sup> observed maximal amounts of estrogenic hormone in the blood between the 7th day preceding menstruation and the onset of the period, and that there was least immediately after menstruation following which the concentration began to rise. It may be, therefore, that a rise in estrogenic hormone in the blood is accompanied, in some women, by a stimulation of the antibody-producing cells, the resulting increased titre outlasting the increase in hormone. When we injected amniotin‡ simultaneously with Type 1 pneumococcus into male rabbits there resulted a somewhat greater concentration of agglutinins than in rabbits receiving the vaccine alone, following the first 3 injections of organisms. This difference was not, however, maintained on further treatment.

Is the season of the year causally related to the fluctuations? Graesser and Harrison<sup>9</sup> found that the pneumococcal power of the sera of 7 of 34 normal adults showed a drop against Type 1 between the fall of 1931 and the spring of 1932. Of these 7, two also showed a drop against Type 2. There were no increases in any of the 34. Ward<sup>10</sup> found no change in pneumococcal power of whole blood in 9 out of 10 persons when tests were done from time to time. No mention is made of the sex of the subjects by these authors. Orr, MacLeod and Mackie<sup>11</sup> observed, in sheep sera, a seasonal fluctuation in bacteriolytic effect on *B. coli*, and agglutinin for *B. abortus* and *B. paratyphosus* B. Pritchett<sup>12</sup> observed seasonal variations in the mortality of mice following *per os* administration of *B. pestis* *caviae*. Season could hardly have influenced our data because the last 4 determinations were made within a period of 2 months. Furthermore, since L.P.'s serum had a high concentration of protective antibody on May 6, 1935, and no antibody on May 1, 1933, and A.E.T.'s blood protected well on April 4, 1932, but not at all on

<sup>7</sup> Frank, R. T., and Salmon, N. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1237.

<sup>8</sup> Frank, R. T., and Goldberger, M. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1663.

‡ Estrus-producing hormone kindly supplied by Dr. John F. Anderson of E. R. Squibb & Sons.

<sup>9</sup> Graesser, J. B., and Harrison, M. C., *J. Exp. Med.*, 1933, **58**, 245.

<sup>10</sup> Ward, K., *J. Exp. Med.*, 1930, **51**, 675.

<sup>11</sup> Orr, J. B., MacLeod, J. J. R., and Mackie, T. J., *Lancet*, 1931, **220**, 1177.

<sup>12</sup> Pritchett, I. W., *J. Exp. Med.*, 1926, **43**, 173.

March 29, 1935, it is unlikely that the fluctuations are related to changes in seasons.

Gaskell<sup>13</sup> reported that the virulence of pneumococci varies with the season. It can be seen from the table that the fatal dose for mice remained fairly constant during the January to June, 1935 tests and that high and low values for antibody bore no relation to this dose. Hence a fluctuation in virulence could not have been the cause of the observed differences.

It is necessary to consider a further explanation. Goodner and Horsfall<sup>14</sup> showed that lipids (cholesterol, lecithin, cephalin) are adsorbed by the immune precipitate when antipneumococcal serum and the capsular polysaccharide are mixed. Their experiments indicate that the addition of cholesterol to a mixture of antipneumococcal serum and Type 1 pneumococci lowers the protective power of horse serum and probably also of rabbit serum. It has been reported by Ikeda<sup>15</sup> that during menstruation the total cholesterol of the blood drops, and that 5 days later the values have not quite reached their normal level. This is the period during which we observed high protective values. Although there is, as yet, no evidence that cholesterol acts with human serum containing protective antibody as it does with antipneumococcal animal sera, it is necessary to keep these data in mind in attempting to arrive at a more detailed explanation for the present results.

It is of interest to note that several investigators have found that serum-globulin rises sharply during menstruation. This is the constituent with which protective antibody is associated in therapeutic sera.

*Summary.* The concentration of Type 2 pneumococcal protective antibody in the serum of 2 women fluctuated during the menstrual cycle, being greater just before, during and shortly after menses and less after the 12th day of the cycle.

We wish to record our appreciation to Dr. Wm. H. Park, through whose courtesy in providing the necessary facilities we were able to carry out these experiments.

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<sup>13</sup> Gaskell, J. F., *J. Path. and Bact.*, 1927, **30**, 518.

<sup>14</sup> Goodner, K., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1936, **64**, 377.

<sup>15</sup> Ikeda, M., *Jap. J. Obst. and Gyn.*, 1932, **15**, 480.