changes would be classed under the term "accidental involution." They occurred in rats each of which received 10 mg of choline hydrochloride per day.

14630

Effect of the Endotoxin of *Shigella paradysenteriae* on Pregnancy in Rabbits.

PAUL A. ZAHL AND CLARA BJERKNES.
From the Haskins Laboratories, New York City.

We have previously reported that the endotoxins of certain gram-negative bacteria, when injected into mice at middle and late pregnancy, induce decidua-placental hemorrhage which often leads to abortion or resorption of the embryos.[1] This hemorrhage induction was considered to be analogous to that induced in implanted mouse tumors by the endotoxins of essentially all gram-negative bacteria. The present study was undertaken to ascertain the effect of such endotoxins on pregnancy in rabbits.

Methods. The acetone precipitate of the residue of dialyzed whole cultures of *Shigella paradysenteriæ* (Flexner) was chosen as containing a representative O antigen of Boivin-type, the principal endotoxin of most gram-negative bacteria. Methods of culture, preparation and standardization are described elsewhere.[2]

The experimental procedure consisted of the intraperitoneal injection into rabbits at various stages of pregnancy of an arbitrarily selected sublethal dose of the Flexner extract. This dose (16 mg) of relatively unpurified material was equivalent to 2 LD_{50} for 20 g white mice or about one-fifth of that lethal to rabbits of 3-4 kg. This high sensitivity of rabbits to the endotoxin of gram-negative bacteria has been noted by other investigators.[3]

The female Rockland rabbits used in this study were multiply mated, a procedure assuring 80-100% true pregnancies. Rabbits so mated received intraperitoneal injections of the endotoxin-containing extract at one of the following stages of pregnancy: (1) 48 hours after mating, *i.e.*, after ovulation and fertilization, but before uterine implantation, (2) 6-7 days after mating, *i.e.*, during the implantation period, (3) 11-12 days after mating, *i.e.*, post-implantation and during development of placental structures, (4) 15-16 days and (5) 19-21 days after mating, *i.e.*, during active growth of embryos and placental tissues, and (6) 25-26 days after mating, *i.e.*, approaching term. Following injection, the animals were observed daily for vaginal bleeding or for evidence of abortion. They were either allowed to proceed to term, or were sacrificed 5 to 10 days after injection, when post-mortem examination was made of the reproductive organs and embryonic tissues. If the embryos and associated membranes appeared to be normal in such sacrificed animals, it was assumed that pregnancy had not been impaired or interrupted.

Results. Of 6 animals injected 48 hours after mating, 5 failed to show further signs of pregnancy. The sixth delivered a normal litter. Of the animals injected at the 6-7-day stage, only one out of 7 underwent interruption of pregnancy. At the 11-12-day stage, 2 out of 4 animals had litters. In the 4 animals at the 15-16-day stage and in the 7 animals at the 19-21-day stage there was uniform interruption of pregnancy although 2 animals of the latter group died within 4 days after injection. At the late stage of 25-26 days 2 out of 9 rabbits delivered normal litters. Among the remaining 7, 3 aborted and 4 died, possibly

[1] Zahl, Paul A., and Bjerknes, C., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 329.

[2] Zahl, Paul A., Hutner, S. H., and Cooper, F. S., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 48; 1943, **54**, 187; Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, 1944, **39**, 189.

[3] Grasset, E., Zoutendyke, A., and Schaafsma, A., *Brit. J. Exp. Path.*, 1935, **16**, 454; Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.*, 1935, **118**, 614.

from toxemia arising from an inability to evacuate dead or injured fetuses. Rabbits injected at stages beyond 15 days often displayed vaginal bleeding and abortion of embryos. In other animals, at post-mortem, dead or resorbing embryos were often found as nodular masses of tissue in the uterine horns. The habit of some rabbits to eat fetuses aborted at night made precise observation in this connection uncertain. A relatively uniform sequel to the injection, usually within the first 24 hours, was profuse diarrhea.

We have been unable to identify the precise site of the hemorrhage but have generally supposed it to occur in the decidua-placental tissues. The aborted fetuses themselves showed no sign of purpura or other evidences of hemorrhage, and the normal rabbit placenta is so highly vascular a tissue as to render observation of induced hemorrhage difficult.

As in the mouse, we observed no sign of hemorrhage or purpura in the ovary and its component tissues, in the adrenals, nor in the normal and pseudo-pregnant uterus. Altnough none of the rabbits used in this study were rebred, mice in which abortion had been induced by the injection of the endotoxin have consistently given birth to normal litters following rebreeding. This would seem to indicate that the dose of endotoxin employed to induce abortion had no permanently injurious effect on the tissues associated with reproduction.

Discussion. The assumption that decidual and placental capillaries, like those of rapidly growing tumors, have a much lower threshold of damage than most other body tissues to vascular poisons of the type found in gram-negative bacteria seems valid as an explanation of the effect of such toxins on middle and late pregnancy when vascular growth is rapid and extensive in the accessory embryonic structures, but it does not explain the interruption of pregnancy during the earlier stages, before implantation or before extensive vascularization has occurred. An alternative explanation for the latter effect may be derived from the thermal changes which occur in the rabbit almost immediately following injection of the endotoxin. The fever curve in Fig. 1 is typical of the thermal changes which occur in the rabbit given the dose of endotoxin by the route of injection employed in this study. Within a few hours after injection the rabbit exhibits a hyperthermia, usually attaining a maximum of 41-42°C within 3 to 6 hours, followed by a slow return to normal during the next 20 hours. That such fever in rabbits is produced by the endotoxins of other gram-negative bacteria is supported by a considerable body of work, representative reports of which are those by Welch *et al.*[4] and by Co Tui.[5]

Cameron[6] has shown that fever (42-43°C) induced by partial submersion in warm water for 20 minutes will interrupt pregnancy in rabbits mated 72-80 hours previously, although, as pointed out by Cameron, artificial fever is generally regarded clinically as a safe form of therapy in middle or late pregnancy. Cameron interprets this as an indication that the rabbit blastocyst has low viability at the fever temperatures indicated. In view of Cameron's findings, it seems reasonable to suggest in connection with the data reported here that the fever induced by the bacterial endotoxin caused death to the blastocyst, thus accounting for the interruption of pregnancy during the pre-implantation stage.

The somewhat unexpected failure of the endotoxin to interrupt pregnancy at the 6-7-day stage could yield to the corollary explanation that the embryo at this stage has overcome its thermosensitivity, and that there has been as yet no extensive vascular infiltration between placental and decidual tissues on which the hemorrhagic factor of the endotoxin could operate. Even if some vascular damage did occur in the decidual tissues, it would not necessarily result in atrophic conditions fatal to the embryos. However, some days after the 6-7-day stage, when vascularization has become definite and well established, it appears that the endotoxin gives rise (as denoted by frequent instances of vaginal bleeding) to hemorrhage in the rapidly growing decidua-placental tissues, causing severe vascular im-

4 Welch, H., Calvery, H. O., McClosky, W. T., and Price, C. W., *J. Am. Pharm. Assn.*, 1943, **32**, 65.

5 Co Tui, *J. Lab. and Clin. Med.*, 1944, **29**, 58.

6 Cameron, J. A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 76.

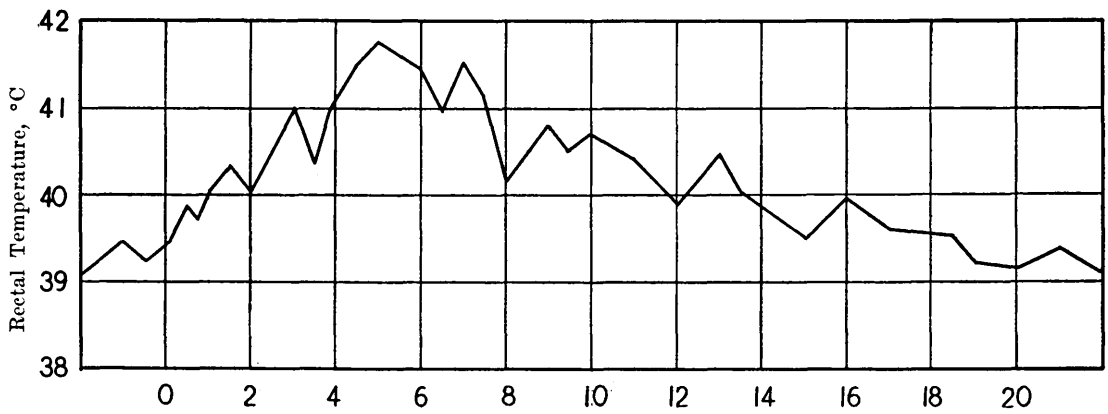

FIG. 1.
Typical temperature chart for female rabbit receiving intraperitoneal injection of ⅕ MLD of the endotoxin of *Shigella paradysenteriæ* (Flexner).

pairment, and leading to resorption or abortion.

Sanarelli[7] states that cholera in pregnant women almost always leads to abortion, and that vibrios injected into pregnant rabbits also give rise to abortion even in the absence of an active infection. Jennings and Mathieson[8] report that abortion or premature labor occurs in 40-60% of pregnant women with typhoid fever infection. They note further that when typhoid infection occurs in the early months of pregnancy, abortion is accompanied by severe hemorrhage. It is to be pointed out that both *Vibrio comma (cholerœ)* and organisms of the typhoid group are gram-negative bacteria whose principal toxic factor is the Boivin-type of endotoxin common to the organisms used by us and, indeed, to most gram-negative bacteria. In previous surveys[9] this type of endotoxin has been shown, almost without exception, to be capable of inducing hemorrhage in implanted mouse tumors.

Summary. Relatively large sublethal doses of the endotoxin-containing extract of *Shigella paradysenteriæ* (Flexner) were injected intraperitoneally into rabbits at the 2, 6-7, 11-12, 15-16, 19-21, and 25-26-day stages of pregnancy. Pregnancy at the 2-day stage was interrupted following such injection, possibly because of a low viability of the rabbit blastocyst to the marked hyperthermia which develops following the injection. The failure of the endotoxin to interrupt pregnancy at the 6-7-day stage is discussed. At stages between the 11th and 26th day, pregnancy was interrupted, accompanied often by vaginal bleeding and abortion which resulted presumably from the hemorrhagic action of the endotoxin on fragile capillaries of the decidua-placental tissues.

This decidua-placental hemorrhage in rabbits is construed to be an action physiologically analogous to the hemorrhage induced by the endotoxins of gram-negative bacteria in implanted mouse tumors.

[7] Sanarelli, G., *Ann. Inst. Pasteur*, 1924, **38**, 11.

[8] Jennings, A. F., and Mathieson, D. R., *Practice of Medicine*, 1940, W. F. Prior Co., Hagerstown, Md.

[9] Zahl, Paul A., Hutner, S. H., Spitz, S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224; Hutner, S. H., and Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 364.