bile, but the intestines showed no color that would indicate the presence of bile.

The results of the experiment are shown in Table I. It is apparent that 0.1 mg of bile salts per gram body weight resulted in the appearance of a much greater degree of hemorrhage in the fasted animals, and that even one-tenth this amount had a definite effect.

Summary. 1. Sudden reduction of atmospheric pressure to 70 mm Hg caused pulmonary hemorrhage and death, in mice. 2. Fasting had the effect of reducing the degree of hemorrhage. 3. Bile salts, given to the fasted animals increased the degree of hemorrhage.

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Induction of Decidua-Placental Hemorrhage in Mice by the Endotoxins of Certain Gram-Negative Bacteria.

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Goldman[1] observed that trypan blue intravenously injected localized in parts of the placenta and in certain of the foetal membranes of mice, but not in the embryos. These observations were in general confirmed by Burrows[2] and by Everett.[3] Paralleling these reports many studies have indicated that colloidal acid-azo dyes in the same class as trypan blue, when injected intravenously, localize in traumatized areas, as well as in certain induced or naturally-occurring lesions. Among these studies was the observation that such dyes localize in neoplasms.

Zahl and Waters[4] made a histological study of the localization of various dyes in mouse sarcoma 180, extending similar earlier observations by Ludford,[5] Duran-Reynals,[6] Brunschwig, Schmitz and Clarke.[7] In the course of this study it was observed that certain bacterial products would, when injected into mice bearing implanted sarcoma, induce hemorrhage of the tumor, often leading to its regression.

Initiated by this observation, a series of studies by Zahl, Hutner and co-workers[8] demonstrated that the endotoxins of almost all gram-negative bacteria when injected into tumor-bearing mice would induce tumor hemorrhage, and that this effect was consistent with the view that endotoxins of gram-negative bacteria are primarily vascular poisons.

Stemming from the apparent resemblance between the localization of colloidal dyes in tumors and the localization of such dyes in placental structures, the following study was undertaken to determine whether a parallel also existed in respect to hemorrhage induction in tumors and hemorrhage induction in placental tissues of mice following injection of the endotoxins of gram-negative bacteria.

Experimental. Three representative gram-negative bacterial species were selected as sources of endotoxin.* These were *Shigella*

1 Goldman, E. E., *Beitr. z. klin. Chir.*, 1909, **64**, 192.

2 Burrows, Harold, *Some Factors in the Localization of Disease in the Body*, Bailliere, Tindall and Cox, London, 1932.

3 Everett, J. W., *J. Exp. Zool.*, 1935, **70**, 243.

4 Zahl, Paul A., and Waters, L. L., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 304.

5 Ludford, R. J., *Proc. Roy. Soc. London*, Ser. B, 1929, **104**, 493.

6 Duran-Reynals, F., *Am. J. Cancer*, 1939, **35**, 98.

7 Brunschwig, A., Schmitz, R. L., and Clarke, T. H., *Arch. Path.*, 1940, **30**, 902.

8 Zahl, Paul A., Hutner, S. H., Spitz, S., Suguira, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224; Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285; Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 116; Hutner, S. H., and Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 364.

* As many workers now agree that the toxic effects of extracts of gram-negative bacteria are

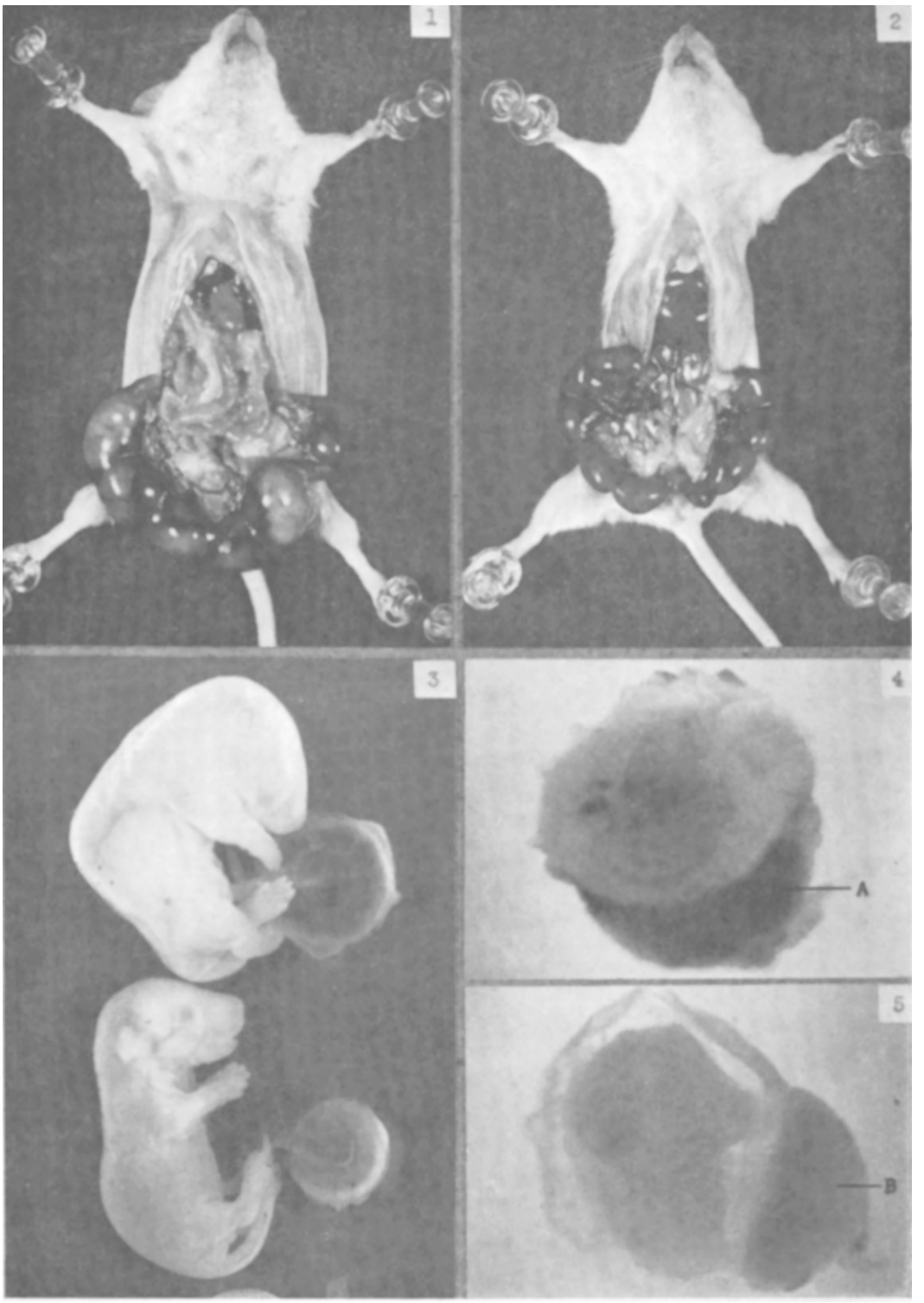
1
2
3
4
A
5
B

PLATE 1.

FIG. 1. Pregnant mouse at 18-day stage, sacrificed 5 hours after injection of 1/5 LD50 of *Shigella* extract. Uterus exposed to show hemorrhagic areas. Three embryos from left horn had aborted before time of sacrifice.

FIG. 2. Pregnant mouse at 15-day stage, sacrificed 5 hours after injection of 1/5 LD50 of *Shigella* extract. Uterus exposed to show general hemorrhagic condition.

FIG. 3. Embryos at 18-day stage. Upper figure is of an aborted fetus from mouse pictured in Fig. 1. Note hemorrhage and general purpura in placental discs. Lower figure is of a normal embryo.

FIGS. 4 and 5. Thick unstained sections through 15-day embryos *in situ* in uterus. Fig. 4 shows embryo from female sacrificed four hours after being injected with *Shigella* extract. Fig. 5 shows embryo from normal pregnant female. Note hemorrhagic (A) and non-hemorrhagic (B) placental discs.

paradysenteriæ Flexner, *Salmonella typhimurium*, and *Rhodospirillum rubrum*. The culture and extraction technics, as well as dose-survival curves for each of the three extracts are published in detail elsewhere.[9]

The effect of these endotoxin-containing extracts on mouse placental tissues was studied by injecting an arbitrarily fixed dose of 1/5 LD50. This dose was lethal to none of the mice. About 150 female mice were injected intraperitoneally with the *Shigella* preparation at pregnancy stages corresponding to 12, 15, and 18 days. Length of the gestation was determined by the vaginal plug method.

Within 8 hours following the injection of endotoxin, pregnant mice showed external vaginal bleeding. Occasionally mice failed to show vaginal bleeding, but upon sacrifice and dissection, free blood was to be found in the lumen of the uterus. It appeared that the nearer mice were to term, the more rapid and profuse was the vaginal bleeding.

Mice between 12 days and term showed detachment of the placental disc and evacuation of the embryos from the uterus within 24 hours. Again it appeared that the closer to term such mice were, the more rapid and complete was the abortion. Mice of the 18-day period often aborted within 4 hours after the injection. Mice of the 12- and 15-day stages sometimes exhibited abortion of only a few embryos, the remainder being retained in the uterus and there presumably subjected to slow resorption. In no instance did pregnant mice injected at the stages indicated produce living young. The effect of bacterial endotoxins on stages of pregnancy earlier than 12 days is being studied.

For testing the *Salmonella* and *Rhodospirillum* extracts only 10 animals each were used, these being chosen at about the 15-day stage of pregnancy. These two extracts were tested largely for the purpose of determining whether the effect observed for one gram-negative form would hold for other gram-negative forms not closely related to the first, and thus to ascertain if the hemorrhagic effect on placental tissues would fall into the class of a general effect found to hold for the endotoxins of almost all gram-negative bacteria in respect to hemorrhage induction in implanted mouse tumors. Decidua-placental hemorrhage was induced regularly by the injection of *Salmonella* and *Rhodospirillum* extracts, and was not essentially different from that induced by the *Shigella* extract.

Following the injection of the three endotoxin-containing extracts, the placental disc and the decidual tissues appeared hemorrhagic or purpural in dissected specimens (see Plate I). Such hemorrhage in these tissues apparently resulted in the release of the placental disc from the wall of the uterus, followed by evacuation of the embryos, or by their resorption. At no time was hemorrhage observed in the embryos. Histological observations on such hemorrhagic tissue will be published elsewhere. It has not as yet been ascertained whether hemorrhage appears in such membranes as the yolk sac epithelium, or Reichert's membrane, where dye localization occurs.

Effect of Bacterial Endotoxins on Other Tissues. It is well established that in the guinea pig the adrenal cortex becomes hemorrhagic following injection of the endotoxins of typhoid and other gram-negative bacteria.[10] We could find no evidence of adrenal hemor-

referable to the endotoxin, the use of this term to describe the active component of our extracts is not considered to involve a liberty in terminology. See reference 9 for detailed discussion of this matter.

9 Zahl, Paul A., and Hutner, S. H., *Am. J. Hyg.*, in press.

rhage in the mice injected with our bacterial extracts. This is in agreement with Cameron, Delafield, and Wilson[11] who failed to find hemorrhage induction in the mouse adrenal cortex following injection of *Salmonella typhimurium* extract.

It was of particular interest to determine whether the ovary and its component parts exhibited a vascular sensitivity to the endotoxins. In no animals, normal or pregnant, did we observe evidence of abnormal hemorrhage in follicles, corpora lutea, or stroma following injection of bacterial endotoxins; nor did we observe hemorrhage in the normal or the pseudopregnant uterus.

Discussion. It is to be noted that in the last half of pregnancy a dual placenta exists in rats and mice.[3] In addition to the obvious discoidal placenta, there is a villated, vascularized yolk sac placenta. Both of these placental structures constitute barriers or are selectively permeable to variously sized colloidal particles. It is the reaction to the bacterial endotoxins of one or both of these barrier tissues, or of the contiguous uterine tissues, which presumably gives rise to hemorrhage.

Kahler, Shear, and Hartwell[12] have recently reported the molecular weight of the hemorrhagic factor of *B. prodigiosus* to be about 8,000,000. The significance of molecular or of colloidal particle size in this connection cannot be evaluated until more is known about the actual mechanism (1) of colloid passage through the living membrance, (2) of epithelial breakdown by the endotoxin. Furthermore, Boivin[13] in subjecting the purified endotoxin (as O antigen) of *Salmonella typhi* to ultracentrifugation found that both the sedimented and non-sedimented fractions were antigenic and toxic. This would indicate that the activity of such material may be associated with particles of widely varying size. That this applies to the material studied by Kahler, Shear, and Hartwell is indicated by Boivin and Mesrobeanu[14] who have demonstrated a close chemical and physiological similarity between the endotoxins of the species (*S. typhi*) on which they made their centrifugation studies and the species (*B. prodigiosus*) used by Kahler, Shear, and Hartwell. Molecular weight determinations on such materials appear, therefore, in this connection, to be of dubious significance.

Hemorrhage in tumors following injection of the endotoxins of various gram-negative bacteria occurs within 3 to 8 hours.[8] The decidua-placental hemorrhage described in this paper occurs within about that same time interval. This, together with the parallelism in dye localization, suggests strongly that these two hemorrhage phenomena fall into the same class of physiological action.

It has been previously recorded that with relatively unpurified bacterial preparations the minimum dose for hemorrhage induction in mouse sarcoma 180 constitutes about 1/200 minimum lethal dose. In highly purified endotoxin preparations the fraction of the minimum lethal dose necessary to induce tumor hemorrhage may become as low as 1/7000.[8] Whether a comparable fraction of the minimum lethal dose is adequate to induce decidua-placental hemorrhage has not yet been determined. Experiments to ascertain this, as well as to ascertain the effect of bacterial endotoxins on the placental structures of higher mammals, are in progress.

Summary. Endotoxin-containing extracts of *Shigella paradysenteriæ* Flexner, *Salmonella typhimurium*, and *Rhodospirillum rubrum* when injected intraperitoneally into mice pregnant from 12 to 18 days induce decidua-placental hemorrhage which results either in abortion or resorption of the embryos.

It is pointed out that such decidua-placental hemorrhage induced by the endotoxins of gram-negative bacteria may fall into the same class of physiological action as hemorrhage induced in implanted tumors following administration of endotoxins of gram-negative bacteria.

10 Olitsky, L., Averiny, S., and Koch, P. K., *J. Immunol.*, 1942, **45**, 237.

11 Cameron, G. R., Delafield, M. E., and Wilson, J., *J. Path. and Bact.*, 1940, **51**, 223.

12 Kahler, H., Shear, M. J., and Hartwell, J. L., *J. Nat. Cancer Inst.*, 1943, **4**, 123.

13 Boivin, A., *C. R. Acad. Sci.*, 1939, **209**, 416.

14 Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.*, 1937, **126**, 652.