the existence of a response.

A definite hematological response should be obvious in reference to either the anemic level or the normal blood picture. The spontaneous variations during the anemic period and the indefinite nature of reticulocyte and red blood cell increases following liver therapy makes this method impractical as a means of bioassay.

Summary and Conclusions. 1. Internal biliary fistulae were produced in dogs. 2. A low grade anemia, mildly macrocytic and irregularly hyperchromic appeared. 3. The parenteral administration of clinically standardized liver extracts elicited no hematopoietic response. 4. This approach is of no value as a bioassay procedure for the Antipernicious Anemia Principle in liver extract.

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Action of Bacterial Toxins on Tumors. V. Immunological Protection Against Tumor Hemorrhage.

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We have found, as similarly reported for *Salmonella typhi*,[1,2] *Salmonella typhimurium*[3] and *Shigella dysenteriae*[4] that the endotoxin of *Shigella paradysenteriae* Flexner when repeatedly administered parenterally to mice confers a definite although limited protection (4 to 6 LD50)* against subsequent doses of the toxin. The object of the present study was to determine whether tumors transplanted into mice previously immunized in the above manner are immune to hemorrhage following injection of Flexner endotoxin in amounts which regularly produce hemorrhage in tumors carried by non-immunized animals. A simultaneous protection against both lethality and tumor-hemorrhage conferred by previous immunizing treatment with the bacterial product would provide an independent test of the hypothesis elaborated in earlier papers of this series, to the effect that lethality as well as tumor-hemorrhage production are both manifestations of the vascular damage characteristic of the endotoxins of gram negative bacteria.[5,6,7]

White Rockland mice were immunized with successive doses of a *Shigella paradysenteriae* endotoxin preparation. Doses of one-half to an LD50 were injected intraperitoneally on 5 successive days, followed on the sixth day by a dose of one LD50. The endotoxin was prepared by growing *Shigella para-*

[1] Favorite, G. O., and Morgan, H. R., *J. Clin. Invest.*, 1942, **21**, 589.

[2] Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.*, 1938, **128**, 835.

[3] Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 116.

[4] Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.*, 1938, **128**, 447.

* In conformity with current practice, we have abandoned the expression "MLD" in favor of the term "LD50" as indicating the dose lethal within 24 hours to 50% of the animals. Likewise, the term "HD50" indicates the dose which will produce definite tumor hemorrhage in 50% of the test animals.

[5] Zahl, Paul A., Hutner, S. H., Spitz S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.

[6] Zahl, Paul A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 285.

[7] Hutner, S. H., and Zahl, Paul A., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 364.

TABLE I.
Tumor Hemorrhage in Immunized and Non-Immunized Mice.

Amt of toxic material inj.	Immunized tumor-bearing mice (degree of hemorrhage)*	Non-immunized tumor-bearing mice (degree of hemorrhage)*
LD50/400†	0 0 0 0 0 0 0	0 0 0 0 0 0
LD50/200	0 0 0 0 0 0 0 0	0 0 0 0 0 ++ + + +
LD50/80	0 0 0 0 0 0 0	+++ +++ +++ ++ ++ ++ + +
LD50/40	0 0 0 0 0 0 0 0 0	+++ +++ +++ +++ ++ ++ ++ ++ ++
LD50/4	0 0 0 0 0 0 0 0 0 0	+++ +++ +++ +++ ++ ++ +
1 LD50	0 0 0 0 0 0 + + + + ++	+++ +++ +++ +++ +++ ++ ++ ++ + +
4 LD50‡	+++ +++ +++ +++ +++ ++ ++ ++ ++ ++	+++ +++ +++ +++ ++ ++ ++ ++ ++
6 LD50‡	+++ +++ +++ +++ +++ ++ ++ ++ ++	+++ +++ +++ +++ +++ +++ ++ ++ ++

Degree of hemorrhage:
0 = no hemorrhage
+ = slight hemorrhage
++ = considerable hemorrhage
+++ = marked hemorrhage
* Each designation below represents one animal.
† The amount of dry toxic dysentery material necessary to produce death within 24 hours of 50% of non-immunized mice injected intraperitoneally was 10 mg. This is therefore designated as one LD50. Four normal LD50 or 40 mg of the toxic material was necessary to produce death in 50% of the immunized mice.
‡ Non-immunized animals at these dosages all succumbed to the lethal effects of the toxic material, but hemorrhage of the tumor was evident before death.

dysenteriae Flexner in liquid medium.† The identity and smoothness of the culture was confirmed by agglutination tests against a standard immune typing serum. After suitable growth the cultures were killed with phenol and the volume reduced by pervaporation through cellulose tubing, followed by precipitation with acetone according to the procedure previously described.[3] Animals immunized with this material were able to survive 4 to 6 LD50 doses of the same preparation on the tenth day after the first immunizing injection.

Ten to 15 days following the first injection, Sarcoma 180 was implanted into mice, using the standard trocar technic. Animals bearing 7-day-old tumors, together with a parallel control series of non-immunized tumor-bearing mice, were given intraperitoneal doses of the same Flexner preparation used for the immunization. Six hours after injection the tumors were examined and graded as to degree of hemorrhage. The results are listed in Table I. It is seen that in the control (non-immunized) animals hemorrhage was induced at all doses greater than 1/200 of an LD50. This 1:200 ratio between the lowest effective hemorrhage and lethal doses is almost identical to the ratio found to hold for the unpurified acetone precipitate of *Salmonella typhimurium* previously re-

† In addition to the usual inorganic salts, the medium contained:
(1) Nicotinic acid—0.04 mg%
(2) Na_3 citrate · $2H_2O$—0.1%
(3) Dextrose—2% (added aseptically)
(4) Casein acid hydrolysate diffusate—0.8%
The diffusate was prepared by dialyzing through cellulose tubing neutralized H_2SO_4-casein hydrolysate. The medium was devoid of high molecular weight compounds, and was therefore considered to be antigen-free.

ported.[3] ‡ For the immunized animals the ratio between HD50 and LD50 is in the order of 1:1, all doses smaller than one LD50 failing to produce hemorrhage. It is evident that immunization with the endotoxin preparation used protects against both tumor-hemorrhage induction and primary lethality; also that the relative effectiveness of immunization is very much greater in the case of hemorrhage induction than in the case of primary lethality.

After immunization the HD50 of the antigen is of about the same magnitude as the LD50, yet for the non-immunized tumor-bearing animals the ratio between HD50 and LD50 is 1:200. Hence it would seem that the neutralizing action of the antibody must be prompt and complete, since but a very small quantity of uncombined antigen would be sufficient to induce tumor hemorrhage. In this respect the protection provided by immunization appears to differ from that afforded by sulfanilamide in that immunization protects against two hundred HD50, whereas sulfanilamide protects against only sixteen HD50.[6] Evidently, and as one would suppose, the mode of antitoxic action of sulfanilamide is of a different nature from the immunological mode of protection.

The preceding papers of this series suggest a uniformity in constitution and in toxic action of the complex O antigens of nearly all gram negative bacteria. The widespread occurrence among gram negative bacteria of the tumor-hemorrhage incitant and its correlation with the occurrence of O antigens was adduced as evidence for the existence of a single uniform toxin in the O antigens of gram negative bacteria.

The present paper describes the type of antitoxic immunity obtained when the same organism is used for both immunization and hemorrhage production. Cross-immunization experiments, using gram negative organisms unrelated except for the exhibition of common tumor hemorrhage production—a highly sensitive method of detecting these endotoxins—is expected to furnish critical evidence as to the unitary character of these toxins. Such experiments are now in progress.

Conclusion. Tumors implanted in mice previously immunized with *Shigella paradysenteriae* Flexner were found, in contrast to those implanted in non-immunized control mice, to be highly resistant to the tumor-hemorrhage factor of the Flexner organism.

‡ It is to be noted that in the case of highly purified or possibly dissociated antigen the HD50/LD50 dosage ratio may be as great as 1:5000.[3,8]

[8] Shear, M. J., *Cancer Research*, 1941, **1**, 731.

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Activity of Parathyroid Hormone in the Nephrectomized Rat.

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The question of whether some of the effects of parathyroid extract injections are producible in the absence of the kidneys has been studied by several investigators, in the effort to decide if parathormone acts primarily upon the kidneys.

Collip and others[1] found that the injection of 80 units of PTH into bilaterally nephrectomized rats produced the customary changes in the bones.

This has been confirmed and the observation recently extended by Selye[2] who showed that nephrectomy alone produces a mild degree of "osteitis fibrosa" which was prevented by parathyroidectomy.

[1] Collip, J. B., Pugsley, L. I., Selye, H., and Thomson, D. L., *Brit. J. Exp. Path.*, 1934, **15**, 335.

[2] Selye, H., *Arch. Path.*, 1942, **34**, 625.