## The Internal Biliary Fistula Dog Unsuitable as a Bioassay Animal for Liver Extract.\*

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Crandall, Finne, and Smith<sup>1</sup> reported a pernicious anemia-like state in dogs with internal biliary fistulae. This anemia was described as macrocytic and hyperchromic, characterized by spontaneous remissions with reticulocyte showers. Single intramuscular injections of 30 U.S.P. units of liver extract in 6 animals resulted in reticulocyte increases of 2-20% and a return of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHb) to within 2 standard deviations of the normal mean. Frequent injections totaling more than 30 units resulted in increases in the red blood count of a million or more cells. The present study is an attempt to confirm the work of Crandall's group and to evaluate its potentialities as an experimental bioassay method for the antipernicious anemia principle in liver extract.

Standard internal biliary fistulae were produced in dogs by suturing the gall bladder into the right renal pelvis; the common bile duct was ligated and sectioned. U. S. Bureau of Standards equipment was used in the erythrocyte counts. Hemoglobin (Newcomer method), reticulocyte (wet method), and hematocrit (Van Allen) determinations were also made. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHb) were calculated. Plasma bilirubin, chloride, non-protein nitrogen, and protein determinations were made at intervals during the experimental period. All animals were autopsied and examined for possible patent bile duct connections to the intestine. Histological sections of bone marrow and other tissues were prepared.

The dogs were fed a standard diet of

canned dog food. Fat soluble vitamins<sup>†</sup> were administered parenterally in adequate doses throughout the experimental period.

*Results.* A mild anemia appeared in 4 out of 7 dogs approximately 4-6 months after operation; data on one of these dogs were discarded since autopsy revealed a patent probably anomalous duct between the gall bladder and intestine. The anemia which appeared may be described as mildly macrocytic and irregularly hyperchromic. The anemia in all dogs was characterized by spontaneous remissions and relapses (Fig. 1) not associated with reticulocyte showers.

Blood chemistry values including the Van den Bergh Reaction were essentially normal during the post-operative period.

Three animals were given synthetic vitamins of the B complex intravenously in excess of the required daily dose for a period of one week. This was done to make certain that the animals were not deficient in these vitamins before testing for a response to liver extract. No hematopoietic response was observed.

In order to test for a hematopoietic response to liver extract, two clinically standardized preparations<sup>‡</sup> (15 U.S.P. units/cc) were administered parenterally to 3 dogs in doses totaling 30-60 units.

No statistically significant hematological responses were observed over and above the anemic level. Fig. 2 indicates the lack of response in one of the dogs.

Bone marrow sections did not reveal the megaloblastic bone marrow characteristic of pernicious anemia.

Discussion. The variation in the anemic

<sup>\*</sup> The investigation was aided by a grant from the Armour Research Fund of the University of Chicago.

<sup>&</sup>lt;sup>1</sup> Crandall, L. A., Finne, C. O., and Smith, P. W., Science, 1941, 93, 549.

t Ephynal acetate obtained through the kindness of Hoffmann-La Roche, Inc.

<sup>‡</sup> Armour Liver Extract (generously furnished by Armour Laboratories); Lilly Liver Extract, Purified.



level was such that 3 Standard Deviations  $\pm$ the mean yielded values above the normal R.B.C. and below the normal MCV. Under such conditions it would be impossible to obtain a statistically significant response. This fact is a practical objection to the method if a response is to be judged in reference to the variation in values obtained during the non-treatment period of the anemia. Since neither a significant reticulocyte response nor a maintained rise in the red blood count was shown by the dogs receiving liver therapy, it seems unlikely that any response took place. The absence of a pernicious anemia-like bone marrow helps to explain the lack of a response of the peripheral blood picture to liver therapy.

Crandall's criterion of a response is the return of the anemic MCV and MCHb to within 2 S.D. of the normal mean without reference to the degree of variation in these values during the anemic period. Although changes in MCV and MCHb took place in his animals following liver therapy, there were no clear cut responses in red blood cell and reticulocyte counts. In view of the spontaneous remissions in the anemic blood picture, it would seem that definite and consistent reticulocyte and erythrocyte increases should be included in the criteria to assure 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,<sup>1,2</sup> Salmonella typhimurium<sup>3</sup> and Shigella dysenteriae<sup>4</sup> 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

<sup>3</sup> Zahl, Paul A., and Hutner, S. H., PROC. Soc. EXP. BIOL. AND MED., 1943, **52**, 116.

<sup>4</sup> 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. 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.<sup>5,6,7</sup>

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*-

<sup>&</sup>lt;sup>1</sup> Favorite, G. O., and Morgan, H. R., J. Clin. Invest., 1942, 21, 589.

<sup>&</sup>lt;sup>2</sup> Boivin, A., and Mesrobeanu, L., C. R. Soc. Biol., 1938, **128**, 835.

<sup>&</sup>lt;sup>5</sup> Zahl, Paul A., Hutner, S. H., Spitz S., Sugiura, K., and Cooper, F. S., *Am. J. Hyg.*, 1942, **36**, 224.

<sup>&</sup>lt;sup>6</sup>Zahl, Paul A., and Hutner, S. H., PROC. Soc. EXP. BIOL. AND MED., 1942, **51**, 285.

<sup>&</sup>lt;sup>7</sup> Hutner, S. H., and Zahl, Paul A., PRoc. Soc. EXP. BIOL. AND MED., 1943, **52**, 364.