TABLE I.
Results Obtained with Injections of Alfalfa Extracts.†
The dosage is expressed in terms of the equivalent of dry, crude alfalfa meal, one kilo being the equivalent of one entire extract.

Duration of alkaline extraction hr.	Extract	Dose in gm.	Ovarian response
4	1	400	+
	1	600	+
	2	750	+
	3	400	+
	3	300	+
	3	300	+
	2	250	—
24	4	750	—
	5	500	—
	5	500	—
	6	500	—
	6	400	—
	7	900	—
	8	800	—
	9	600	—

† The successful extracts were prepared from 3 separate shipments of alfalfa which differed considerably in color, odor, texture, and protein content.

injections of a variety of materials, including male urine and sterol suspensions, serve adequately as controls.

It is apparent, then, that gonad-stimulating extracts can be prepared from alfalfa. The active material may, or may not, be related to the pituitary hormone, and these data may or may not have direct physiological significance, although they are in harmony with the working hypothesis which stimulated this work.

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Experimental Studies in So-called Agranulocytic Angina. I. Effects upon Leucocytes by Toxic Products of Bacteria from Stool of Human Case.

WILLIAM H. HARRIS AND HERBERT J. SCHATTENBERG.

*From the Department of Pathology, School of Medicine, Tulane University.**

The possible causal relationship of the intestinal bacterial flora in agranulocytic angina has not been investigated. We carried out bacteriological examinations of stool specimens of a human case

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and recovered together with other of the intestinal microorganisms, cultures of *B. enteritidis, Streptococcus hemolyticus* and *B. welchii.* This paper deals with the results of animal inoculation with these cultures.

B. enteritidis. Six guinea pigs were injected, 2 subcutaneously, 2 intraperitoneally, and 2 intracardially, with 1 cc. each, of a saline suspension of this strain. Neutrophilic leucocytosis resulted and the animals died in 24 to 72 hours.

Three guinea pigs were fed with oats contaminated with stools containing *B. enteritidis,* and over the food of 3 guinea pigs suspensions of recovered culture were poured. After 6 weeks, including repeated leucocytic counts, no appreciable effects of such feedings were noted.

Two guinea pigs were injected, 1 intracardially and 1 intraperitoneally with the filtrate of broth cultures and increase of total and granulocytic leucocytes resulted.

In previous work upon production of lesions of experimental typhoid fever in the guinea pig with *in vivo* prepared toxic products of *B. typhosus,*[1, 2] it had been noted that a marked leukopenia resulted. No observations upon the granulocytes were made. We employed this method in the present work. A fatal peritonitis is primarily produced and the resultant exudative materials including toxins and saline washings of the peritoneal cavity were collected and filtered through a Berkefeld or Seitz-Wertz filter. A modified toxic moiety is procurable. Fifteen guinea pigs were injected by this method, employing the *B. enteritidis* isolation. The toxic filtrate was administered in doses of 1 to 5 cc. subcutaneously, intraperitoneally and intracardially. The intraperitoneal and intracardial routes and the larger doses were more effective. In all animals, a distinct lowering of the total leucocytic counts and a proportionately greater reduction of the granulocytes were observed. The period at which the greatest decline occurred averaged 72 hours with ordinarily an increment in the first 24 hours. The count of 1 animal injected intracardially is seen in the chart. Reductions of from 57% to 73% of the total and of from 90% to 96% of the granulocytes occurred.

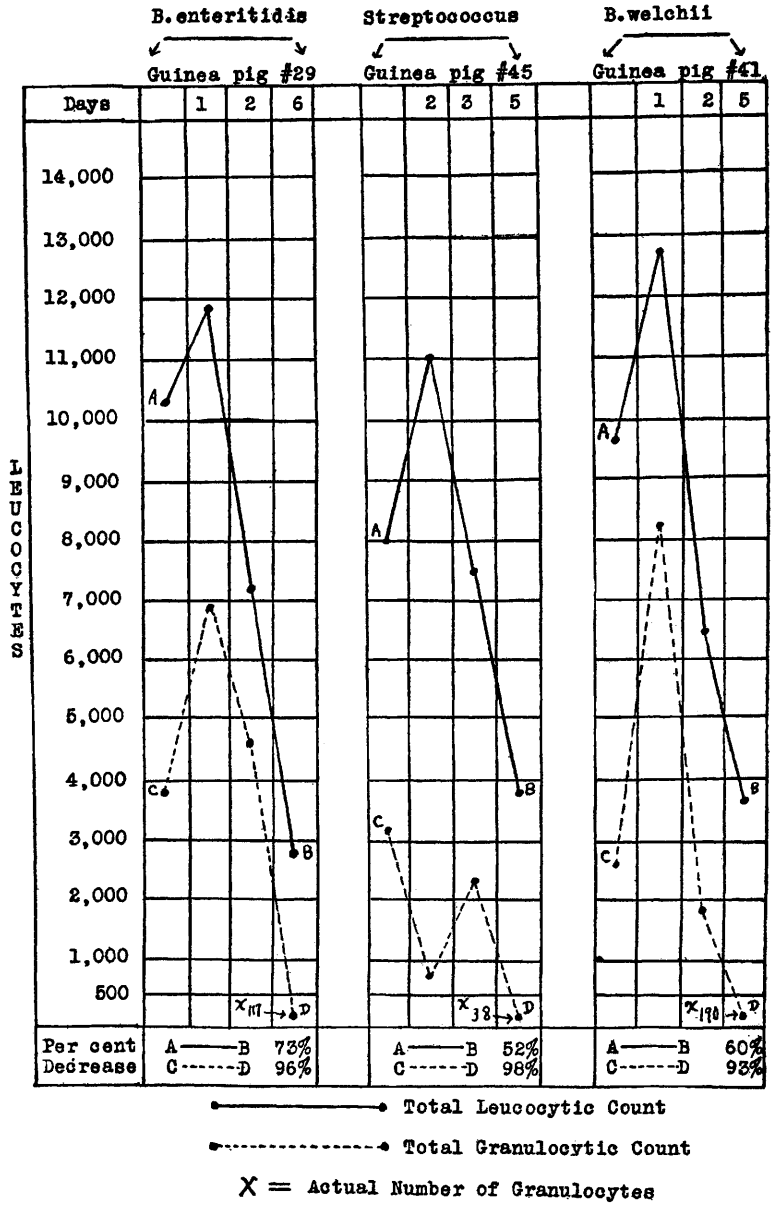
Streptococcus hemolyticus. The inoculation of this culture and of filtrates of the broth culture subcutaneously, intraperitoneally and intracardially into guinea pigs yielded neutrophilic leucocytosis. *In vivo* prepared toxic products were obtained by the intraperitoneal

[1] Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 372.

[2] Harris, W. H., and Larimore, O. M., *J. Exp. Med.*, 1928, **48**, 885.

CHART 1.

Effects of Bacterial Toxic Products upon Leucocytes.



injection of this culture into the guinea pig. The Seitz-Wertz filtrate of the peritoneal exudate and washings was injected intraperitoneally into 2 guinea pigs, 45 and 46 in doses of 5 cc., and intracardially into guinea pigs 43 and 44, each receiving 3 cc. Consider-

able reduction of the total leucocytic count with marked reduction of the granulocytes was produced (see chart).

B. welchii. The whey portion of the litmus milk cultures was filtered through a Seitz-Wertz filter. This filtrate in amounts of from 2 to 10 cc. was introduced daily for 2 weeks, through a tube, into the stomach of 6 rabbits. No effect upon the general well being of these animals or upon the leucocytic counts was noted. The introduction of the living culture in this manner into 4 rabbits showed no change. This toxic filtrate of the whey portion of the culture was administered into 2 rabbits intravenously and 9 guinea pigs intracardially. Each rabbit was given 2 doses intravenously of 1 cc. at 6-day intervals. The total leucocytic count was usually increased following these injections, with a corresponding increase of the granulocytes but after 72 hours, the total count returned to normal. The granulocytes, however, were reduced from an average of approximately 40% to amounts as low as 5% following a primary rise but later returned to normal. The guinea pigs injected intracardially with this toxic filtrate in doses of more than 1 cc. succumbed within 24 hours. In the animals inoculated with 0.5 cc., the leucocytic and granulocytic curves were the same as in the rabbit, *i. e.,* primary rise of both with subsequent return of the total count but diminution of the granulocytes to between 5 and 8%.

Four guinea pigs were injected intraperitoneally with 3 to 5 cc. of the *B. welchii* filtrate and these animals yielded marked reductions of both the total and granulocytic counts. The leucocytic findings of a representative animal are shown in the chart.

It is to be noted that for this culture the *in vitro* toxin was sufficient to produce the leucocytic depressions and that no *in vivo* prepared toxic material was necessary.

Repetition of the injections of the toxic factors of these microorganisms that produce granulocytopenic leukopenia, lead to lowered resistance and death of the animals in 2 to 4 weeks, often from secondary invaders; this commonly occurs in the human case.

It is shown in these experiments that the *in vivo* prepared toxin of *B. enteritidis* and *Streptococcus hemolyticus* and the *in vitro* toxic filtrate of *B. welchii* will produce the blood picture found in the human case of so-called agranulocytic angina.