data, it is apparent that whole activated pancreas, unlike pancreatic juice, contains a thermo-labile material which can reduce by 50% both viscosity and ability to form fibers of acid soluble collagen solutions. This activity is not affected by various trypsin inhibitors, nor is it duplicated by trypsin, elastase, chymotrypsin or hyaluronidase alone. This activity may be due to a new enzyme in the pancreas, a collagenase. In view of lack of activity of this enzyme upon serum and egg albumin, casein and gelatin, this enzyme would seem to be reasonably specific for acid soluble collagen.

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Mobilization of Neutrophils into Peritoneal Fluid Following Intraperitoneal Injection of Bacterial Endotoxins.* (25272)

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Contrary to frequent reports that a 0.9% NaCl solution provokes local neutrophilia when injected intraperitoneally, we found that this does not occur in rats if the saline solution is sterile and non-pyrogenic. Moreover, a solution to which a small amount of a bacterial polysaccharide complex derived from Pseudomonas (Piromen) had been added, produced a marked neutrophilia when injected intraperitoneally(1). Therefore, it was of interest to determine whether or not cellular reactions of peritoneal fluid can be used as a sensitive indicator to detect presence of small amounts of certain bacterial substances.

Methods. Male rats of Sprague-Dawley strain (Holtzman Co.) and weighing 180-220 g were used. All rats received an intraperitoneal injection of 10 ml sterile, non-pyrogenic saline (Baxter Lab.). Solutions were injected at room temperature and the rats allowed food and water *ad lib.* during experiment. Animals were challenged by graded amounts of bacterial extracts added to the saline solutions immediately before injection. Three bacterial extracts were used: 1) Piromen,† a Pseudomonas polysaccharide complex (Baxter Lab.), 2) Staphylococcus A lipopolysaccharide (Difco Lab.), and 3) *E. coli* 026: B6 lipopolysaccharide (Difco Lab.). Control animals received only sterile saline solution, free of pyrogens. After 5 hours rats were sacrificed, then 10 ml heparinized 0.9% saline solution was injected intraperitoneally. This was done because most of the original 10 ml of solution is absorbed from the cavity by 5 hrs. Animal's abdomens were massaged gently for one minute. Then an incision was made into the peritoneal cavity and the fluid containing a suspension of cells was collected with medicine dropper. The fluid was centrifuged to concentrate the cells which were then resuspended in homologous serum. Smears were made using a brush technic(2), and stained by modified Wright-Giemsa stain(3). In the unstimulated state the peritoneal fluid contains mononuclear cells (macrophages and lymphocytes), eosinophils and mast cells, but is virtually devoid of neutrophils. Percentages of various cells, excluding mast cells, were computed. On the basis of previous experience, it was decided that appearance of 5% or more neutrophils in the peritoneal fluid

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TABLE I. Appearance of Neutrophils in Peritoneal Fluid 5 Hours after Injection of Bacterial Extracts.

Treatment	Dose, μg/rat	Positive response/total No. of rats
Piromen	Saline control	0/16
	1×10^{-4}	0/9
	$5 \times$ "	0/4
	1×10^{-3}	6/12
	Graded doses from 3.16×10^{-3} to 3.16×10	91/92
Staphylococcus A polysaccharide	Saline control	0/8
	1×10^{-5}	0/4
	1×10^{-4}	4/8
	$5 \times$ "	5/7
	Graded doses from 1×10^{-3} to 1×10^{4}	31/31
E. coli 026:B6 polysaccharide	Saline control	0/4
	5×10^{-4}	0/4
	Graded doses from 1×10^{-3} to 1×10^{3}	28/28

should constitute a positive response.

Results. Table I summarizes effects following injection of graded doses of the 3 bacterial derivatives used. Both Piromen and *E. coli* extracts evoked a measurable response following injection of .001 μg of material, and Staphylococcus A extract was potent when as little as .0001 μg was injected. Control animals which received injections of 10 ml sterile, non-pyrogenic saline were all negative in regard to neutrophil mobilization. Once a dose necessary to elicit a maximum response was reached, practically all animals responded irrespective of dose. The neutrophils which appeared in the fluid were mature and resemble those seen in circulating blood.

Discussion. The massing of neutrophils in the peritoneal cavity following injection of as little as .0001 μg of a bacterial lipopolysaccharide offers proof of the extreme sensitivity of the rat to certain bacterial derivatives. This is even more remarkable since the rat has been thought to be singularly unresponsive to such substances(4). The official method for detection of certain bacterial contaminants in solutions is based on the febrile response in rabbits(5). Unfortunately, the rabbit has a labile thermo-regulatory mechanism and frequently gives false positive results(6). The alteration of peripheral white blood cell count also has been suggested as a means of detecting pyrogens in fluids(7). Some investigators have called attention to the peripheral leukopenia, noted shortly after injection of pyrogen(8) while other authors noted, in addition, that pyrogenic substances evoke a neutrophilic leukocytosis several hours after their administration(9). The need for serial white blood cell counts coupled with the normally fluctuating nature of the leukocytic picture constitute difficulties in assessing such changes. In contrast, examination of peritoneal fluid offers some distinct advantages over previous methods. Since the unstimulated animal has practically no neutrophils in the peritoneal cavity, their presence there, even in small numbers, is quickly detected and easily quantitated. Secondly, one can inject relatively larger volumes of fluid intraperitoneally than intravenously or subcutaneously. Also, only a single determination/animal is necessary. Preliminary studies showed that the neutrophil response can be tested in other laboratory animals such as the mouse. It will be necessary to test the response to many types of substances to ascertain specificity of the peritoneal fluid response to bacterial products. Such work is now in progress. Glucose, for example, in contrast to published observations, was ineffective in mobilizing neutrophils in the peritoneal fluid(10), provided it was administered in pyrogen free solutions.

Although our report is concerned with percentage changes in neutrophils only, quantitative studies involving absolute cell counts have been done and will be reported. These studies reveal that very high doses of bacterial polysaccharides may diminish total numbers of neutrophils which can be mobilized, although the response is not abolished entirely.

Since the neutrophil plays a primary role in defense of the organism against certain bacteria, it is perhaps not surprising that one can stimulate the mobilization of these cells by such minute amounts of bacterial material. Moreover, the rationale for using mobilization of neutrophils to detect and assess bacterial products gains additional support from the work of investigators showed the close relationship between neutrophils and pyrogens (11,12). It will be of interest to determine

whether peritoneal fluid response can be equated with febrile response.

Summary. Injection of certain bacterial polysaccharides into the peritoneal cavity of rats results in a local neutrophilia which can easily be determined and quantitated. Preliminary findings suggest the possibility of using this response to detect the presence of certain bacterial derivatives in solutions.

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Thyroiditis Resulting from Administration of Excess Iodine to Hamsters with Hyperplastic Goiters.* (25273)

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When physiological amounts of iodine are administered to hamsters which have hyperplastic goiters produced by an iodine-deficient diet(1), colloid rapidly accumulates in the follicles to bring about the morphological picture of colloid goiter. The suitability of the hamster for studies on the thyroid prompted us to investigate the effects of large amounts of iodine in order to reproduce, if possible, the state of *Jodbasedow.* It will be recalled that T. Kocher(2) introduced this term in 1910 to refer to the syndrome of clinical hyperthyroidism occurring in persons having endemic goiters to whom iodine had been administered. The only clear-cut example of this phenomenon in the experimental animal was reported by Webster and Chesney(3) 30 years ago when they administered large amounts of iodine to rabbits with hyperplastic goiters produced by a cabbage diet. Such animals lost weight, developed increased metabolic rates, and died; at autopsy their thyroid glands exhibited a patchy reaccumulation of colloid, but not true colloid goiter. When hamsters with hyperplastic iodine-deficient goiters were given large amounts of iodine, as will be reported herein, an inflammatory reaction developed in the gland, giving rise in a short time to the picture of sub-acute thyroiditis. It is the purpose of this communication to describe the evolution of this process in hyperplastic glands produced by iodine-deficiency or goitrogens(4).

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Materials and methods. A total of 28 hamsters weighing approximately 100 g were observed on 3 different diets: 1) an iodine-deficient ration composed of whole ground corn, 76; wheat gluten, 20; calcium carbonate, 2; and sodium chloride, 2; and 500 mg of a complete vitamin mixture per kilo. 2) thiouracil and 3) potassium thiocyanate, added to batches of the basal diet at levels of .3% and .5%, respectively. Sixteen animals were maintained on the iodine-deficient diet for periods up to 30 weeks. The thiouracil-containing diet was administered to 6 animals for 3-6 weeks, while potassium thiocyanate was