its application to other bacterial infections, must await future experimentation. Landy and Pillemer have shown protection against *Pseudomonas* and other Gram-negative bacterial infections by prior injection into mice of lipopolysaccharides that increase the properdin titre of blood serum(5). However, properdin is reported to be in Fraction III (Cohn) of the plasma proteins(5), while γ globulin is obtained from Fraction II. Likewise, heating serum for 30 minutes at 56° destroys properdin titre(5), while in our experiments with plasma no loss of activity occurred.

Summary. A factor in the γ globulin fraction of human plasma has been shown to prevent death in mice from *Ps. aeruginosa* infections, when injected subsequent to the inoculation. Protection was demonstrated in normal mice, and in mice rendered more susceptible to this infection by pretreatment with cortisone, or by subjecting them to thermal trauma. Human serum albumin was without activity.

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Tissue Heparin and Mast Cells in Rats and Rabbits.* (22905)

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It is generally assumed that heparin is synthesized and stored in the mast cells, and that variations of mast cell numbers and of heparin contents are parallel, when different tissues are compared(1,2,3). Recently species differences in susceptibility to atherosclerosis were correlated with differences in mast cell numbers; the greater resistance to this disease of certain species such as, *e.g.*, the rat, was attributed to heparin being available in relatively greater quantities, since this anticoagulant was shown to accelerate removal of certain plasma lipids, via the clearing factor(4). To test this hypothesis, tissue heparin concentrations and mast cell numbers were compared in species resistant and susceptible to atheromatosis, *i.e.*, the rat and rabbit.

Material and methods. Twenty adult male rats of the University of S. California strain, fed Purina Chow, and 12 adult male albino rabbits obtained from a commercial breeder, were used without further treatment. The animals were anesthetized with nembutal, and liver, intestine, kidneys, lungs, spleen and thymus were excised and weighed. From each of these organs, a small slice was removed for histological examination; samples of 1-5 g were then taken for heparin extraction, weighed and stored in the frozen state. Heparin was extracted, defatted, deproteinized by tryptic digestion and partially purified according to a method adapted to relatively small samples, and relatively low heparin concentrations, as reported recently (5). The heparin content of the extracts obtained was measured in line with a semimicro

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modification of the procedure described in the U.S. Pharmacopeia XIV. Duplicate samples were run on many of the larger organs, in particular, those derived from rabbits. In the case of small organs such as spleen and thymus, however, samples of several animals were usually pooled.

The combination of extraction and assay procedures described here is considered to be specific for heparin, since the determination is based on anticoagulant activity, and as no other known naturally occurring anticoagulant can survive the treatment involved, as far as we know. To further establish the identity of the heparin obtained, a number of characteristic tests were carried out, including protamine-reversal of anticoagulant activity, paper electrophoresis, and metachromatic activity of the product obtained in the latter procedure. According to these criteria, the heparin of the tissue samples was identical with a reference preparation of heparin sodium USP, as reported recently(5). For microscopic examination, tissue samples were fixed in a mixture of 1 part formalin and 2 parts 80% (v/v) alcohol, embedded in paraffin and cut at 5 μ. Fixation of rabbit material with basic lead acetate proved unnecessary. Various basic dyes were tried for staining. For routine use 1% aqueous toluidine blue was chosen. Rat sections were stained for 10-15 minutes, those of rabbits for 15 minutes; the slides were then quickly dehydrated with isopropylalcohol. Eight to 10 days later, metachromasia was well developed. Most of the rat mast cells turned purplish-black; a few remained pink. Very tightly packed granula, *e.g.*, in the elongated mast cells of the thymus capsule, appeared even greenish-black. On rabbit tissues, nearly all mast cells stained pink. Mast cell counts were made on 20 optical fields under objective 40 and ocular 7; this corresponds to a total area of approximately 3.5 mm^2. When the distribution of the mast cells was very irregular, or cells laden with soot or hemosiderin rendered counting difficult, the counts were repeated several times. Pink lumps in the rabbit kidney were counted as full cells, even though they possibly were cell fragments.

TABLE I. Heparin Content and Mast Cell Count of Rat Organs.

Organ	No. of rats*	Mean heparin content: No. of measurements	Mean heparin content: Units/g	Mean mast cell count, No./3.5 mm^2
Liver	19;20	19	1.73± .12‡	10.8±1.6‡
Spleen	20;19	8†	4.84± .35	3.6±1.4
Intestine	20;20	18	5.55± .58	12.5±4.2
Lung	20;19	9†	6.32±1.04	17.2±1.7
Kidney	18;19	15†	12.84±1.69	3.5± .9
Thymus	19;16	2†	13.3	66.2±9.5

* First figure, heparin assay; second figure, mast cell count.

† The smaller organs of 2 to 4, and, in the case of thymus, of 9 and 10 animals, were pooled for heparin assay; the stand. error was computed for No. of measurements indicated.

‡ ± stand. error.

TABLE II. Heparin Content and Mast Cell Count of Rabbit Organs.

Organ	No. of rabbits*	Mean heparin content, units/g	Mean mast cell count, No./3.5 mm^2
Liver	12;11	1.11 ± .14†	28.7 ± 4.4†
Thymus	10;12	1.34 ± .23	21.8 ± 3.8
Intestine	12;12	2.80 ± .35	42.3 ± 3.0
Spleen	11;11	3.50 ± .49	25.2 ± 5.4
Lung	12;12	5.01 ± .92	30.9 ± 5.6
Kidney	12;12	5.22 ± .81	27.5 ± 4.5

* First figure, heparin assay; second figure, mast cell count.

† ± stand. error.

Results. Table I summarizes the results of heparin and mast cell determinations on rat organs. Thymus and kidney showed the highest, and liver the lowest heparin contents, of the tissues examined. The latter finding was unexpected, for in other species, *e.g.*, the dog, the liver is very rich in heparin, and the anticoagulant was first isolated from, and named for this organ. Intestine, lung and spleen contained moderate amounts of heparin. Variations among individual animals were greatest in the case of the kidney and lung.

The observation on rat mast cells agreed with the reports of earlier workers(6,7). The cells varied in size, and many were very large. On liver sections, mast cells aggregated around a few portal veins but were scarce in the main mass of stroma. The high mast cell content of the thymus and the surrounding fat and mesothelia was associated with signs

of thymus involution. In the lung, healthy areas contained a moderate number of mast cells, and portions, where the alveoli were partially closed by proliferating cells, very few. In the intestine the regional differences in mast cell number were very great; as stated by other workers the intestinal mast cells have polymorphous nuclei like basophilic leucocytes. No mast cells were seen in 4 kidney and 11 spleen samples (out of 19).

Heparin contents and mast cell numbers showed a similar distribution pattern only for some of the rat organs tested, *i.e.*, thymus, lung and intestine. In the liver, on the other hand, an intermediate count was associated with a low heparin level. Kidney and spleen showed practically identical mast cell numbers (the lowest of the group), though the former contained about 2½ times as much heparin as the latter.

In the rabbit, the kidney and lung showed the greatest, and liver and thymus the lowest heparin contents of the tissues examined. In all cases, the values were lower than those of the corresponding rat organs, but the pattern of distribution over the different organs was similar in these two species, with exception of the thymus. Regional variations in heparin content were observed when portions of the same lung or kidney were assayed separately.

The mast cell counts of the different rabbit organs again did not go parallel with their heparin contents. The distribution of cells was more even than in the rat; rabbit tissues exceeded the corresponding rat organs in mast cell numbers, excepting the thymus, but the cells took on only a pale color and were of moderate size. The low number of thymic mast cells might be related to the mildness of involutionary processes.

Discussion. The authors are aware of the difficulties involved in a comparison of tissue heparin studies of different laboratories, since almost every investigator uses different procedures, or, at least, different modifications of certain technics, for extraction and assay of the anticoagulant. Only a few papers in the literature deal with quantitative determinations of tissue heparin levels in rats and rabbits. Wilander and Jorpes observed rat liver to be free of heparin(2), a result not much out of line with the low value found in the present investigation, but indicating that the new method used by the present authors may be more sensitive. Recently, Monkhouse reported values for "extractable heparin" in several rabbit organs significantly lower (about 5 to 100 times) than those presented above(8). This discrepancy might be explained, in part, on the basis of differences in rabbit strain and in assay procedure; the possibility is also considered, however, that the extraction procedure used in the present work is more complete.

The data on the mast cell content of rat organs presented above are in line with results of other workers(6,7). Statements in the literature dealing with rabbit mast cells vary widely. In the present work, relatively high mast cell numbers were observed in rabbit tissues, in agreement with findings of Holmgren and Wilander(1). Michels(9), Constantinides(4), and most earlier authors, on the other hand, reported low mast cell counts in this species. In part, this discrepancy might be explained on the basis of differences in strain and age of the animals. Furthermore, it is conceivable that the fixation was inadequate in some of the early work, since the rabbit mast cell granula are highly soluble.

On the basis of observations by Holmgren and Wilander, Jorpes stated that the stainable material of mast cell granula consists of heparin and that tissue mast cell and "sulphuric ester" content go parallel, the latter being identified with heparin(2). However, when the relationship between tissue heparin and mast cells is studied, not only the cell number, but also the heparin content of the individual cell have to be taken into consideration. Rat mast cells are larger and stain more deeply than those of the rabbit; therefore, individual rat cells contain correspondingly more metachromatic material. These observations might explain why, in the present work, rat tissues yielded more heparin than those of rabbits, although they contained less mast cells. But even when organs of the same species were compared, tissue heparin

and mast cell distributions did not follow the same patterns. If it is assumed that heparin is derived from mast cells, then our results indicate that the distribution of heparin through the body was modified by regional differences, either in the composition of the mast cell granula (proportion of heparin to hyaluronic acid and other mucopolysaccharides), or in the capacity of tissues to retain heparin after its release from the cells.

In the course of the present study, more heparin was found in the tissues of the rat than in the tissues of the rabbit. As the former species exhibits greater resistance to hyperlipemia and atherosclerosis, the observations agree with the theory that the heparin content of the tissues may be one of the factors which contribute to this resistance.

Summary. Tissue heparin contents and mast cell counts were determined on liver, lung, intestine, kidney, spleen and thymus of male rats and rabbits. In most instances, distribution of heparin and mast cells did not follow the same pattern. Rat organs showed higher heparin values, but lower mast cell numbers than the corresponding rabbit tissues.

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Physiological Response of "Insulinase" and Its Inhibitor in the Hypoinsulin State.* (22906)

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Mirsky *et al.*(1-6) have demonstrated a tissue activity in liver capable of inactivating insulin and have named this activity "Insulinase". They also have described the ability of filtrates from heated homogenates of the same livers to inhibit Insulinase. The response of this system of Insulinase and its inhibitor to the level of circulating insulin and its conceivable role in the homeostasis of blood sugar values in the frog and rat have been investigated.

Methods. Intact and depancreatized frogs (*Rana pipiens*) were divided into 2 groups: one group maintained at 23°C and the other at 2-5°C. Pancreatectomy in frogs was accomplished as follows. The frogs were anesthetized with ether and a mid-line abdominal incision was made exposing the pancreas. The major blood vessels of this organ were ligated and cut prior to removal of the pancreas, and the abdomen was then closed in the usual manner. 5-0 silk was used throughout the procedure. The following day the group of normal and operated animals kept at 23°C were force-fed (minced calves liver and bone meal dipped in cod liver oil). After 6-7 days, frogs of both groups were pithed, blood samples were taken by heart puncture for sugar determinations(7), and the livers then extirpated, chilled in ice-water and prepared as indicated below. Male rats of the Holtzman strain weighing 175-200 g were kept in individual wire-bottomed cages and fed a stock diet. Animals were fasted for 65 hours prior to subcutaneous injection of 15 mg of alloxan (Eastman) per 100 g body weight as a 3%

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