nism, possibly in conjunction with the normal "erosive" properties of red blood cell flow. 5. The morphologic characteristics of the injury *in vivo,* and by histologic sectioning have been described. 6. There is evidence that the endothelial cytoplasm may have phagocytic properties under the conditions of the experiments described here.

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Serum Beta-Glucuronidase Activity of Various Species: Possible Correlation with Susceptibility to Atherosclerosis.* (30394)

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Beta-glucuronidase activity has been found to be increased in human atherosclerotic arteries by Dyrbye and Kirk(1) who studied intima-media portions of the aorta and coronary arteries, and also by Branwood and Carr(2) who assayed the intimas of coronary arteries. In cholesterol-induced atherosclerosis in the rabbit, Mrhova *et al*(3) observed that the enzyme activity was also increased in whole aortic extracts. On the other hand, Kayahan(4) reported decreased values for beta-glucuronidase activity in the intima of human, atherosclerotic aortas.

Kayahan noted briefly(4) a high value for

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serum beta-glucuronidase activity in the cat which is resistant to atherosclerosis, and lower serum values in more susceptible species such as the chicken and the rabbit. We have investigated more systematically the serum beta-glucuronidase activity in a number of species of interest in research on atherosclerosis. We have included additional resistant species such as the dog and rat and also some such as the swine and pigeon which are comparable to the human in their susceptibility to atherosclerosis. Comparisons have been included between species which develop atherosclerosis spontaneously with those that require special "induction" techniques. Also, resistant and susceptible strains within a given species have been investigated.

It has been interesting to find that the level of serum beta-glucuronidase activity appears to correlate with susceptibility of a species to develop either spontaneous or induced atherosclerosis. Some of our results have been reported(5).

Methods and materials. To determine the serum beta-glucuronidase activity 2 methods were employed. In the method of Goldbarg *et al*(6) the substrate on which the beta-glucuronidase acts is 6-bromo-2-naphthyl-B-d-glucuronide. The method of Fishman *et al* (7) as modified by Plaice(8) estimates the enzyme activity by its reaction with phenolphthalein-glucuronide. In most instances we employed both methods on the samples of serum. With each method calibration curves were prepared on the serum of each species. Since both methods give different absolute values of enzyme activity depending on the size of the sample, all results are reported on 0.2 ml samples of serum. This variation in enzyme activity appears to be larger than would be expected from a protein adsorption effect, and probably depends on the concentration of inhibitors and activators of the beta-glucuronidase in serum.

In the method of Goldbarg *et al*, a citrate-phosphate buffer, pH 4.95, was found to give more reproducible enzyme assays than the pH 4.5 acetate buffer suggested in the original method.

All blood samples were obtained by intracardiac puncture, venipuncture, or exsanguination depending on the size of the animals under study. The clotted whole blood was immediately centrifuged for 5-10 minutes and the clear supernatant serum removed. The serum was either analyzed immediately or frozen at —5°C for future assay.

The following animals were included in the study: 12 mongrel dogs ranging in age from one to 10 years (20-35 kg); 9 mongrel cats, 9 to 10 weeks old; 9 male Sprague-Dawley rats (300 g); 12 adult male guinea pigs (1.0-1.2 kg); 9 adult Californian rabbits (2.0-2.7 kg); 8 adult Dutch rabbits (2.0-2.7 kg); 6 adult English rabbits (2.0-3.0 kg); 4 adult New Zealand rabbits (3.2-4.3 kg); 9 Rhode Island Red chickens (1.2-1.7 kg); 12 White Highline chickens (1.2-1.7 kg); 5 adult Show Racer pigeons; 10 adult White Carneau pigeons; 12 adult White King pigeons; and 23 swine from 10 weeks to 10 years of age (20-200 kg). Human sera were obtained from patients in the Hospital of the University of Pennsylvania awaiting elective surgical procedures, which varied in nature from inguinal herniorrhaphy to abdominal aneurysmectomy. This group of 10 males and 4 females ranged in age from 7 to 67 years.

Results. The average values of beta-glucuronidase activity in human, swine, pigeons, cats, and dogs are given in Tables I and II. The range of enzyme activity in the sera is also shown for each species. In any one species the range of values is moderately wide. However, repeated determinations of serum enzyme activity in an individual animal gave reproducible results.

Activities obtained by both the Goldbarg and Fishman methods were relatively low in humans, swine, and pigeons when compared with those obtained in sera of cats and dogs.

TABLE I. Serum Beta-Glucuronidase Activity. (Method of Goldbarg *et al*)

Species	No. of Samples	Average Activity*	Activity Range
Human	14	52	4- 104
Swine	13	470	92- 800
Pigeon	25	690	270-1420
Cat	9	2390	1310-3750
Dog	10	2410	1550-4170

* Activity expressed as micrograms of chromogen released per 100 ml of serum per hour of incubation.

TABLE II. Serum Beta-Glucuronidase Activity. (Method of Fishman *et. al*)

Species	No. of Samples	Average Activity*	Activity Range
Human	11	630	190-1550
Swine	17	0	—
Pigeon	25	700	160-1500
Cat	12	2280	1150-3550
Dog	9	2640	1800-3800

* Activity expressed as micrograms of chromogen released per 100 ml of serum per hour of incubation.

By the Goldbarg method human serum activity averaged 52 μg of chromogen released per 100 ml of serum per hour of incubation, as compared to an average value of 470 in swine, 690 in pigeons, 2390 in cats, and 2410 in dogs. Corresponding values by the Fishman method were as follows: humans, 630; swine, 0; pigeons, 700; cats, 2280; and dogs, 2640. The serum enzyme activity in swine is particularly noteworthy since no activity was observed in 17 swine when the Fishman method was employed, although there is significant activity as measured by the method of Goldbarg.

Chicken, rabbit, guinea pig, and rat serum activity (Table III) fell between the very low and the very high levels reported in Tables I and II. No significant differences in serum enzymatic activity were observed between the various strains of pigeons, rabbits, or chickens.

Discussion. In Tables I and II the results show a notable difference in serum beta-glucuronidase activity between the human, pigeon, and swine as compared with the dog and cat. The differences are essentially the same by either the method of Goldbarg *et al* or of Fishman. The most marked difference in enzyme activity appears with the swine sera. Consistently, in the sera of swine the assays for beta-glucuronidase by the Fishman method have given zero values, whereas the assays by the Goldbarg procedure gave low but measurable results. To ensure that some fortuitous error had not been introduced in the assays by the Fishman method, a series of 10 additional sera were analyzed after all reagents were freshly made and the enzyme substrate was replaced by one from a different source. Again, all the enzyme activities were zero in the swine, whereas the fresh reagents and the new substrate gave the expected results on human and dog sera. The absence of activity in swine sera on the phenolphthalein-glucuronide substrate appears to be independent of the pH of the incubation mixture because substantial shifts in pH to both the acid or alkaline side did not produce any measurable beta-glucuronidase activity. Likewise, no activity was elicited by changing the amounts of serum employed in the enzyme assay. Because of the measurable enzyme activity demonstrated in swine serum with the 6-bromo-2-naphthyl-B-d-glucuronide substrate (Goldbarg method), it seems likely that the phenolphthalein-glucuronide substrate is more sensitive to the presence of an endogenous inhibitor in the serum of this species. This point is now being studied.

The marked differences in activity of the serum beta-glucuronidase between man, swine, and pigeons as a group as compared with dogs and cats becomes of interest in the study of atherosclerosis because of the marked differences in susceptibility of the two groupings in these 5 species.

It is admittedly difficult to quantitate "resistance" and "susceptibility" of a given species to atherosclerosis. However, the fairly extensive observations in the literature make it clear that man, swine, and pigeon fall into the susceptible class and that dog and cat constitute 2 species with low susceptibility to *spontaneous* atherosclerosis(9,10,11,12,13,14, 15,16).

It is clear that a relationship exists in this grouping of species between the serum enzyme activity for beta-glucuronidase and the susceptibility of the species in general to

TABLE III. Serum Beta-Glucuronidase Activity.

	Goldbarg method		Fishman method	
Species	No. of Samples	Avg Activity*	No. of Samples	Avg Activity*
Chicken	20	600	20	890
Rabbit	27	760	22	1400
Guinea pig	12	1320	12	1600
Rat	9	2030	0	—

* Activity expressed as micrograms of chromogen released per 100 ml of serum per hour of incubation.

atherosclerosis. However, it should be emphasized that we do not imply a *causal* relationship. The relationship, if it proves to be significant, could conceivably be a mere indicator of another series of causal chemical events in the pathogenesis of atherosclerosis; or possibly the relationship may be fortuitous.

In Table III rabbits and chickens are compared with guinea pigs and rats. In both the rabbit and chicken, atherosclerosis may be induced easily and regularly by feeding cholesterol. In the guinea pig, this type of atherosclerosis may be produced with greater difficulty(17). The rat is extremely resistant to the production of atherosclerosis(18,19). Here, too, the serum beta-glucuronidase activity correlates with the susceptibility of the species to the experimentally induced disease. By the Goldbarg method, the average value for chickens was 600 and for rabbits, 760; the value for the more resistant guinea pigs was 1320 and for the highly resistant rat it averaged 2030. The selection of species in the various Tables is somewhat arbitrary. For example, the dog could have been included with the rat in Table III since cholesterol atherosclerosis can also be produced in this species with special techniques. It is interesting to note that the serum beta-glucuronidase values for the rat and the dog lie close together in the high range.

The relationship between serum beta-glucuronidase activity and susceptibility to spontaneous or induced atherosclerosis appears to hold only for a species as a whole, and not for intra-species differences in susceptibility. For the human we did not find any striking difference in serum enzyme activity between young women and men despite the great contrast in their susceptibility to atherosclerosis. When we analyzed our data for the pigeon we did not find significant differences between the White Carneau and Show Racer breeds despite the marked resistance of the latter strain to atherosclerosis (11). Also, in the rabbits studied we did not observe significant differences in serum beta-glucuronidase activity between the Californian, Dutch, English, and New Zealand strains which have been shown by Gaman(20) to differ in their susceptibility to induced atherosclerosis. In the chickens included in our results no difference was observed between the White Highline strain as compared with the Rhode Island Red. The White Highline is highly susceptible to induced cholesterol atherosclerosis whereas the Rhode Island Red is resistant(21).

It may be that differences within these species will be shown when a more specific method for estimation of serum beta-glucuronidase becomes available, as now seems likely(22).

Summary. Serum beta-glucuronidase activity has been studied in a number of species: man, swine, pigeon, chicken, rabbit, guinea pig, rat, cat, and dog. The values appear to correlate with the susceptibility of the species to spontaneous or induced atherosclerosis.

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Effect of Alloxan on Lactation and Replacement Therapy with Insulin in the Rat.* (30395)

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The influence of graded levels of insulin upon the secretion of milk by the rat from day 14 to 20 of lactation has been reported (1). It was shown that daily injection of 3 units of protamine zinc insulin into rats of the Sprague-Dawley-Rolfsmeyer strain increased milk yield 38% on day 14, 25% on day 16, 26% on day 18 and 79% on day 20. It was suggested that insulin plays a role in the lactation process and that the rate of secretion of insulin in this group of rats was less than optimal.

It has been shown that injection of alloxan at a level of 15 mg/100 g body weight (bw) induces a chronic state of diabetes in our strain of rats(2). It was observed during the 13th to 18th days after the injection that feed consumption increased over 50% above the previous level.

The object of the present study was to determine the effect of an alloxan induced deficiency of insulin upon the milk secretion of rats and, if milk secretion was reduced, to determine the level of insulin which would restore lactation to normal or above.

Materials and methods. Fifty lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, fed Purina lab chow and water *ad libitum*. On day 4 of lactation the litters were reduced to 6, and a single intraperitoneal injection of alloxan (reagent) at a level of 15 mg/100 g bw was administered to induce a chronic state of diabetes(3). Twenty rats treated with alloxan were used as alloxan-treated controls. One-half of the controls were put into metabolism cages to measure feed consumption. Protamine zinc insulin suspension (USP) (Lilly†) was diluted in alkaline saline solution. From day 5 to 19 of lactation the dams were injected subcutaneously, once daily, approximately at the same time as follows: (1) 20 controls were injected with alkaline saline, (2) 20 rats were injected with 2 units of insulin; 5 rats of this group were put into metabolism cages to measure feed consumption, and (3) 10 rats were treated with 3 units of insulin. Milk yields were estimated as previously described(1). On day 20 the dams were killed after nursing, the 6 posterior mammary glands were removed and DNA was determined as described previously (4). The amount of DNA/100 g bw was used as an index of the extent of mammary gland growth.

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