irreversible complex and (2) formation of a micelle. The ability of these agents to induce hyperlipemia in animals was reflected by inhibition of enzymatic lipolysis *in vitro*.

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Increased Urinary Pressor Activity (Aortic Strip Assay) in Tolbutamide-Treated Diabetes.*† (30522)

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Measurements of urinary pressor activity (aortic strip assay) in healthy subjects, in patients with newly diagnosed or known and treated diabetes mellitus, and in non-diabetic adults with symptoms suggestive of endocrinopathy have revealed differences which are statistically significant. The data and possible interpretations are herein presented.

Materials and methods. Pressor activity (aortic strip assay) has been measured in a total of 692 twenty-four hour specimens of urine collected with refrigeration from the same number of subjects and patients. The results are expressed as gamma epinephrine equivalents or g.e.e.(1).

The presence or absence of diabetes mellitus was determined in each subject by means of one or more oral glucose tolerance tests(2).

The results have been grouped into 5 categories: a) healthy adult controls, male and female, b) ambulatory non-diabetic patients with symptoms or signs indicative of possible endocrinopathy, c) adults with newly detected and untreated diabetes, d) adults with known diabetes under diet and insulin treatment, and e) adults with known diabetes under diet and tolbutamide therapy (0.5 to 1.5 g per day) (Table I).

Each subgroup was in turn divided into age categories by successive decades between 20 and 70 years, and then by weight, *i.e.*, 169 lb or less and 170 lb or higher to determine whether these variables were related to urine pressor activity.

The statistical significance of any differences between each of the 4 groups of patients and the control subjects, and within each patient group subdivided as to age and body weight was evaluated by T test ($p<0.05$ indicated statistically significant differences).

Results. Urinary pressor activity in 116 healthy adult subjects ranged between 8.1 and 158.8 g.e.e., averaging 27.8 $\pm$ 27.5 g.e.e. (Table I).

Urinary pressor activity was higher by about one-half in a series of adults with symptoms, signs and laboratory findings indicative of an endocrinopathy or a metabolic disorder other than diabetes mellitus (Table I). This group of 312 patients comprised

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TABLE I. Urinary Pressor Activity (Aortic Strip Assay) in Subjects Without and With Diabetes Mellitus.

Subjects	Including values greater than 100 g.e.e./24 hr Mean ± S.D. (n)	Excluding values greater than 100 g.e.e./24 hr Mean ± S.D. (n)
Healthy controls	27.8 ± 27.5 (116)	23.8 ± 17.4 (112)
Ambulatory patients without diabetes	*35.9 ± 28.8 (312)	*32.4 ± 19.2 (303)
D.M. without Rx	*37.2 ± 32.0 (112)	*33.6 ± 24.3 (107)
D.M. (insulin therapy)	*50.8 ± 75.0 (104)	*33.4 ± 19.5 (97)
D.M. (tolbutamide therapy)	*50.3 ± 68.0 (48)	*32.9 ± 18.4 (44)

* Differences between these and the control values are statistically significant.
g.e.e. = gamma epinephrine equivalents.

104 with obesity, 14 with renal disease, 29 with hypothyroidism, 18 with hyperthyroidism, 18 with collagen disease, 90 with adrenogenital syndrome and/or polycystic ovaries, and 39 with various complaints which could not be ascribed to any endocrinopathy.

In each of these the glucose tolerance test (1.75 g of glucose per os per kilogram of body weight with measurements of the venous blood sugar(2) prior to and at ½, 1, 2, 3, 4, and 5 hours) was non-diabetic within several days of the collection of the 24-hour urine specimen for pressor assay. In each of these patients the fasting blood sugar level was less than 120 mg%, the values at ½ and 1 hour were less than 180 mg%, and the 2-hour and subsequent levels were less than 120 mg%.

The presence of newly diagnosed and untreated diabetes mellitus based on the criteria cited above was associated with an increase (again approximating 50%) in the level of pressor activity in the urine. A greater increase (about 100%) was recorded in known diabetic patients under therapy with diet and tolbutamide or with diet and insulin. The increase in the mean values of pressor activity in these patients resulted largely from the presence of sporadic values in excess of 100 g.e.e. (Table I). These were equally frequent in the insulin and in the tolbutamide treated groups. Recalculation of the mean values in tolbutamide or insulin treated diabetic subjects after exclusion of all values in excess of 100 still yields a mean which is significantly different from the control value (Table I).

Subdivision of the 5 categories of subjects or patients in accord with age by decades or with body weight (using 170 lb as the dividing point) failed to reveal any effect of these 2 variables on the urinary pressor activity recorded for the individual groups as a whole.

Discussion. The higher urinary pressor activity in patients with symptoms or signs which result in a visit to a physician or hospitalization may be the result of stress. The fact that the presence or absence of untreated diabetes does not modify this increase suggests that this finding may be related to illness in general and not to diabetes or other specific disorders.

The increase in the urinary pressor activity in insulin-treated diabetes is well recognized. Obvious or undetected absolute hypoglycemia or even sudden decreases in blood sugar levels evoke a release of epinephrine(3). However, the presence of similar increases in urinary pressor activity in diabetic patients treated with tolbutamide suggests but does not establish that this agent may produce absolute or relative hypoglycemia, or a tendency thereto, more often than one would judge from the sporadic reports of absolute hypoglycemia observed in patients treated with this agent(4-11).

Summary and conclusions. 1. In ambulatory patients with suspected or proved but untreated endocrinopathies including newly-diagnosed but untreated diabetes mellitus urinary pressor activity as measured by an aortic strip assay is about one-half again as great as that recorded in comparable age controls. 2. Therapy of diabetes mellitus with either insulin or tolbutamide is associated with increased urinary pressor activity which is some two-fold higher than that observed

in control subjects. 3. Neither age nor the presence or absence of obesity appeared to be related to the level of pressor activity in the urine in any of the groups studied.

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Mast Cell Degranulation in Fixed Preparations.* (30523)

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Degranulation of mast cells has been reported to occur in response to a variety of pathological and experimental conditions. On the one hand, it was said that it is a *sine qua non* of massive histamine release(1). On the other hand, it has been maintained that histamine release can occur without mast cell degranulation(2,3).

Much of the literature dealing with mast cells is based on studies of histological preparations where it is assumed that fixation precludes subsequent cytological changes. Whereas this may be essentially so for many cell types, we have found that it is not for mast cells after methanol fixation as commonly practiced.

Materials and methods. Rat peritoneal fluid preparations were made either by allowing a spread drop to dry on the slide or by a brush technique(4). When dry, they were fixed in methanol for 10 minutes as commonly done in hematology. Methanol fixation up to 14 days was also used to insure complete fixation. The cells were again air-dried and a coverslip was applied on the dry preparation. The cells were then examined microscopically at magnifications of 400 or 600 times with phase or with interference contrast (Nomarski optics) and individual cells were kept under observation while various reagents were introduced under the coverslip. Basically, the following conditions were examined: a) rehydration of the fixed cells with various aqueous media and b) substitution of the aqueous medium of a) with either aqueous toluidine blue or a saline solution of the histamine liberator Compound 48/80.† Direct measurements of cellular diameters were made with an ocular filar micrometer. Observations on individual granules also were made. Time-lapse cinephotomicrographic recordings were obtained‡ from which rapid changes in individual cells and granules could be followed reliably. The measurements of diameters are not readily translatable into volumes because of the extreme flattening of the cells in these preparations.

Results. Rehydration with distilled water, saline, or dilute aqueous toluidine blue almost

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