Elution mit konzentrierter Schwefelsäure behandelt und das Absorptionsspektrum gemessen. Wie aus der Tabelle zu entnehmen ist, stimmte es mit dem Spektrum des Standards völlig überein.

Auf Grund dieser Kriterien ist die Identität der aus dem Urinextrakt gewonnenen Substanz mit authentischem Testosteronsulfat gesichert. Ein weiterer Teil des Eluates des dritten Papierchromatogrammes wurde mit Dioxan[9] solvolysiert und im System Propylenglykol/Hexan rechromatographiert (Imprägnierung mit 20% Propylenglykol in Methanol, Laufzeit 24 h, Bezugssubstanz Cortexon = 1, Lokalisation durch UV-Kontaktphoto). Als Standardsubstanzen liefen Testosteron und Epitestosteron parallel. Die aus dem Urinextrakt freigesetzte Substanz zeigte den gleichen R_S-Wert (0,59) wie Testosteron. Eine dem Epitestosteron entsprechende Zone liess sich nicht nachweisen (R_S-Wert für Epitestosteron = 0,96). Die den Standards korrespondierenden Urinzonen wurden mit 80%igem Methanol eluiert und im Dünnschichtsystem Chloroform:Methanol (98:2, v/v) auf Silicagel G (Merck, Darmstadt) getrennt rechromatographiert (Rf-Wert für Testosteron und Epitestosteron 0,42 resp. 0,41). Nach Behandlung der Platten mit einem *p*-Toluolsulfonsäure-Spray zeigte sich unter der UV-Lampe nur die typische Grünfluoreszenz des Testosterons, während die charakteristische Rotfluoreszenz des Epitestosterons nicht nachweisbar war.

Diskussion. Während in den Mitteilungen anderer Autoren[3-5] Testosteronsulfat im Urin «indirekt» nachgewiesen wurde, d.h. Testosteron erst nach einem Solvolyseverfahren als freies Steroid identifiziert wurde, konnte in dieser Arbeit erstmalig das konjugierte Steroid als solches charakterisiert werden. Nach der solvolytischen Spaltung wurde nur Testosteron, nicht dagegen Epitestosteron gefunden. Inzwischen wurde auch Epitestosteron-sulfat im menschlichen Urin identifiziert[10]. Die Quantitäten von Testosteron-sulfat im männlichen Urin sind relativ gering. Dessypris et al.[5] fanden 6–10 µg/24 h. Unsere eigenen Werte lagen mit 10–17 µg/24 h geringgradig höher[2,11]. Die im Vergleich zum Testosteron-glucosiduronat niedrige Ausscheidung von Testosteron-sulfat dürfte z.T. auf die sehr niedrige metabolische Clearance-Rate des Sulfatesters zurückzuführen sein[12].

Summary. Testosterone sulphate has been isolated from the urine of a normal male subject. The characterization of the substance was based upon the following criteria: Identical Rf values of authentic testosterone sulphate and of the unknown compound in the paperchromatographic system S1; positive methylene blue-reaction and UV contact photogram; identical absorption spectra in concentrated sulphuric acid. Following dioxan solvolysis, only testosterone has been detected. Epitestosterone could not been found.

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Dose Response of Gastric Secretion, and Electrolyte Output in Pylorus-Ligated Rats to Insulin Hypoglycemia

It is well known that the s.c., i.m. and i.v. administration of insulin provokes gastric acid secretion in a variety of species[1,2]. The exact mechanism(s) of this stimulatory effect is unknown; however, stimulation depends upon an adequate fall in blood sugar, and an intact vagal pathway[1]. Insulin can, under certain circumstances, inhibit gastric secretion in man[3] and dogs[4], and moreover fails to stimulate gastric secretion by hypoglycemia in goats[5]. The close dependence of insulin-stimulated gastric secretion is well documented for dogs[6,7]. However, little attention has been paid to the doses of insulin used in reported experiments with rats, a point which assumes importance, because differing patterns of gastric secretion have been reported following graded insulin dosage in dogs[6]. In the present study, using pylorus-ligated Shay rats[8], attempts have been made to determine maximal gastric acid secretion, the pattern of gastric juice electrolytes, and the relationship between total acidity and the rate (volume) of gastric secretion, following graded doses of insulin.

The animals used were 60 male Sprague-Dawley rats from the Charles River Laboratories breeding shed I, weighing between 200 and 250 g. The rats were randomly divided into 6 groups of 10 rats per group, and, following ligation under light ether anesthesia, were injected s.c. with 2, 4, 6, 8 and 10 U crystalline zinc insulin/100 g body weight. (All rats receiving 12 U insulin/100 g developed convulsions within 2 h.) Control rats received saline injections. 2 h later the rats were rapidly anesthetized with open ether and exsanguinated. (2 h collection periods have been recommended by Brodie[9] and by Baume and Law[10] with pyloric ligation.) The gastric juice was drained into a centrifuge tube, and, following centrifugation, analyzed for gastric acidity and volume, and sodium,

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potassium and chloride content. Details of our preoperative treatment, surgical technique and methods of gastric juice analysis have been reported previously[11]. Blood sugar was estimated by LOEWUS' method[12], blood samples being withdrawn (tail vein) at 0 time, and 1 and 2 h following insulin in 3 rats at each dosage level.

The blood sugar concentration fell at almost the same rate from 0–60 min irrespective of insulin dose; the recovery being proportional to the dosage of insulin used. With 8 and 10 U insulin/100 g the blood sugar fell to between 10 and 15 mg/100 ml at 1 h; values still being below 40 mg/100 ml at 2 h.

Gastric juice volume, gastric juice volume/100 g body weight[13], acidity, acid output, and total acidity as a function of insulin dosage are indicated in Figure 1. Each point represents the mean value of 10 rats. Maximal gastric secretory response occurs in all parameters with 4 U insulin/100 g body weight. However, it should be emphasized that this is not the same thing as 'the maximum gastric secretory response possible', because gastrin can give rise to a greater acid output[14]. This dose of 4 U/100 g is far greater than that reported to give maximal gastric secretory response in dogs[6,7,15], and almost certainly represents a species difference. Parenthetically, it should be remembered that gastric secretion in the rat is far less susceptible to histamine stimulation[11,16] than in man or dogs[17].

The patterns of the gastric juice electrolyte content following graded doses of insulin are indicated in Figure 2. These data fit the 'characteristic' patterns of gastric juice electrolytes following insulin hypoglycemia as reported in dogs[18], particularly the rapid and persistent reduction in the concentration of potassium[19] which in the present report fell from a control level of 17.3 mEq/l to 9.1 mEq/l.

Some of the data presented here support the exchange diffusion hypothesis of TEORELL[20] between sodium and hydrogen ions, as indicated in Figure 3. This curve, which indicates the relationship between the sodium and hydrogen ion concentration in the 5 groups of rats receiving insulin, is highly significant ($P < 0.01$, $r = -0.88$). However, we were unable to confirm the suggestion of LINDNER et al.[21], who maintained that the rate of entry

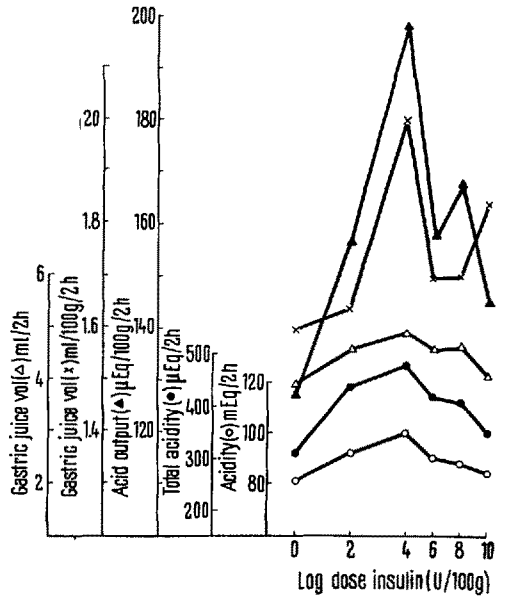

Fig. 1. Dose dependence of gastric juice volume (△), gastric juice volume/100 g body weight (×), acid output (▲), total acidity (●) and acidity (○), to graded doses of insulin. Each point represents the mean value of 10 rats. Maximal gastric secretory response occurs with 4 U insulin/100 g body weight.

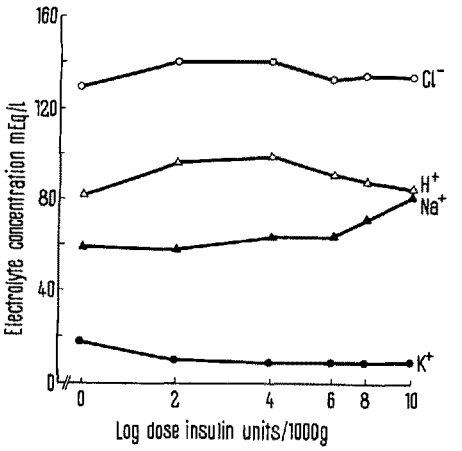

Fig. 2. Dose dependence of gastric juice electrolytes to graded doses of insulin. Each point represents the mean value of 10 rats.

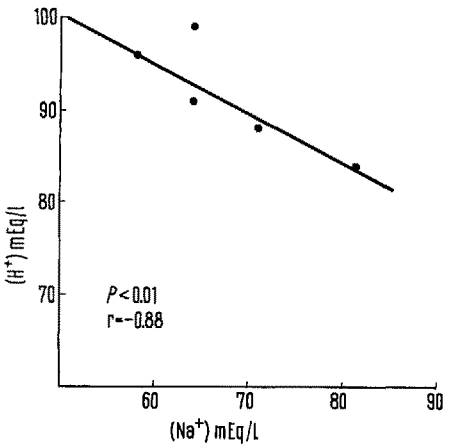

Fig. 3. Regression line demonstrating the relationship between the sodium and hydrogen ion concentration in gastric juice following graded insulin dosage. Each point represents the mean value of 10 rats.

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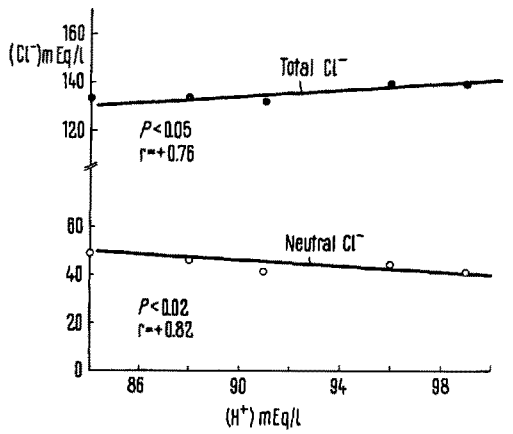

Fig. 4. Regression lines demonstrating the relationships between total and neutral chloride and the hydrogen ion concentration in gastric juice following graded insulin dosage. Each point represents the mean value of 10 rats.

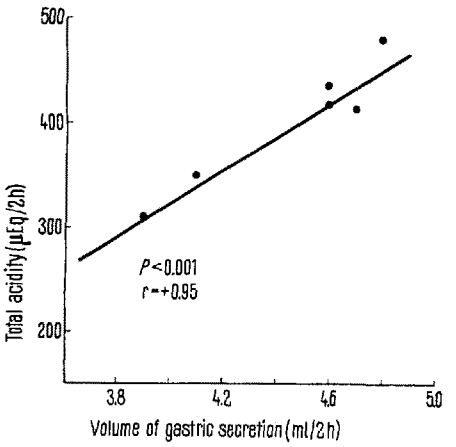

Fig. 5. Regression line demonstrating the relationship between the total acidity and the volume of gastric secretion following graded insulin dosage. Each point represents the mean value of 10 rats.

of potassium ions into the gastric juice was related to the volume rate of secretion. From Figure 4, which represents the relationship between acidity and the total and neutral chloride in the 5 groups of rats receiving insulin, it can be seen that as the acidity increases the concentration of total chloride increases also, whereas the concentration of neutral chloride decreases. This suggests that neutralization-dilution mechanisms (i.e. the 2-component hypothesis of gastric secretion proposed by HOLLANDER[22] in 1932), as well as exchange diffusion, may be operating to produce the rise in gastric acidity following insulin hypoglycemia. Finally, the close relationship between total acidity and the rate (volume) of gastric secretion following graded insulin dosage is indicated in Figure 5. This association is highly significant: $r = + 0.95$, $P < 0.001$.

From the present study it appears that maximal gastric secretory response develops with 4 U crystalline zinc insulin/100 g body weight in pylorus-ligated Shay rats. Furthermore, under graded insulin dosage, patterns of gastric juice electrolyte changes occurred supporting both neutralization-dilution and exchange diffusion mechanisms in the production of gastric secretion[23].

Zusammenfassung. Die Insulinwirkung auf die Magensäuresekretion wurde in männlichen Sprague-Dawley Ratten untersucht, deren Pylorus 2 h vorher unterbunden war. Eine Dosis-Wirkungskurve wurde aufgestellt. Maximale Sekretion von Magensäure und Magensaft wurden durch s.c. Injektion von 4 Einheiten krystallinischen Zink-Insulins per 100 g Körpergewicht erzielt. Grössere Dosen von Insulin unterdrücken Säure- und Magensaftsekretion. Das Verhalten der Elektrolyte im Magensaft spricht sowohl für die Neutralisierungs-Verdünnungshypothese als auch für die Austausch-Diffusionshypothese zur Erklärung der Magensaftproduktion.

Y. H. LEE and J. H. THOMPSON

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Hyperbaric Oxygen and Testicular Damage and Fertility

It has been reported that male rats which survive after exposure to raised pressures of oxygen develop testicular atrophy and signs of defective spermatogenesis[1-3]. In these experiments the diagnosis of testicular damage was based on macroscopic and microscopic appearances. In the report of DE ALMEIDA[1], the experimental conditions of exposure and pressurization of rats was not made clear. In the studies of MATTEO and NAHAS[3], rats were kept continuously for periods of up to 4 weeks enclosed in aquarium tanks through which 98% oxygen at ambient pressure was circulated, the CO_2 produced being absorbed. This treatment caused the death of most rats within the first 5 days, testicular atrophy being seen in survivors. The authors rightly suggest that testicular damage by oxygen may be of importance in the therapeutic applications of inhaling enriched oxygen mixtures at ambient or hyperbaric pressures in man.

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