seems that barring accidents incident to surgery this animal will always or nearly always survive. This conclusion is buttressed by the fact that half and even more of the intercostal nerves can be destroyed in addition without causing death.

Summary. Seven dogs were kept in good health and without respiratory distress for periods varying between 9 and 67 days after bilateral phrenectomy. All of the animals were eventually sacrificed, none died as a result of paralyzing the diaphragm. Electrical stimulation and microscopic examination proved that the phrenectomies were complete in all cases. It was established that following paralysis of the diaphragm at least half of the intercostal nerves, either upper or lower, may be sectioned without loss of the animal. The conclusion drawn is that reports or statements to the effect that the diaphragm is essential for life in otherwise intact grown dogs are erroneous.

1. Brown, J. O., and Satinsky, V. P., *Am. J. Med. Sci.,* 1951, v222, 613.
2. Greene, R., *South. Med. J.,* 1932, v25, 392.
3. Angle, L. W., *ibid.,* 1932, v25, 1012.
4. Campbell, M. F., *Am. J. Surg.,* 1940, v48, 449.
5. Lemon, W. S., *Arch. Surg.,* 1928, v17, 840.
6. Hare, H. A., and Martin, E., *Lancet,* 1890, v1, 124 and 185.
7. Lemon, W. S., *Proc. Staff Meet. Mayo Clinic,* 1928, v3, 161.
8. Jappeli, A., *Boll. d. Soc. Ital. d. Biol. Speriment.,* 1930, v5, 1126.
9. Feldman, M., and Morrison, S., *Am. J. Digest. Dis. and Nutrit.,* 1934, v1, 471.
10. Coombs, H. C., and Pike, F. H., *Am. J. Physiol.,* 1930, v95, 681.

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Effect of Insulin on Maximal Rate of Renal Tubular Uptake of Glucose in Non-Diabetic Humans.* (20634)

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Although the various physiological effects of insulin are well known, the specific mode of action of the hormone has not been clearly delineated. At present, it is generally believed that insulin facilitates, in some manner, the phosphorylation of glucose which introduces carbohydrate into the metabolic processes of the cell. The exchange of glucose between glomerular filtrate and renal tubular cell appears to be a unidirectional process and is characterized by a maximal rate of tubular cell uptake of glucose. Glucose is completely removed from the filtrate until filtered load of glucose is increased to a point where the tubular resorptive mechanism is saturated. Further increments in filtered glucose appear in the urine. This situation would appear to provide a unique opportunity to study the effect of insulin on the rate of cellular uptake of glucose in the intact animal as contrasted to most *in vivo* work which has been concerned with the net exchange of glucose between cell and perfusing medium.

Methods. Twelve male subjects aged 47-68 years who were free of evidence of renal disease, cardiovascular disease, and derangement of carbohydrate metabolism were studied in the fasting state. The general procedure outlined by Goldring and Chasis(1) for the determination of Tm glucose was followed. After suitable priming dose, glucose was infused as a 30% solution in water at a rate of approximately 1.5 g per minute throughout the entire procedure. Inulin was added to the infusion fluid in quantities sufficient to maintain plasma levels of 20-25 mg %. Blood was sampled at the mid-point of each urine collection period through an in-

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TABLE I. Effect of Insulin on Tm Glucose,* mg/min.

Subject	Age, yr	Height, cm	Wt, kg	Control period 1	2	3	Post-insulin period 4	5	6	7	8
L.	64	172	61.7	292	264	255	266	240	220	272	252
S.	48	170	59.9	288	254	282	298	321	261	321	299
B.	47	172	68.5	324	338	401	339	306	272	287	286
D.	65	162	61.0	158	133	126	149	134	116	146	127
B.	68	172	85.6	254	261	243	255	261	189	231	310
McK.	63	172	86.0	227	225	234	210	186	176	174	204
H.	52	172	54.1	217	218	212	215	194	187	232	210
J.	62	178	70.0	289	242	276	278	247	257	270	341
B.	65	165	61.0	341	322	352	390	347	286	306	299
A.H.	47	176	65.1	376	379	377	360	302	350	333	382
C.	65	168	56.8	243	236	256	206	154	217	198	217
F.C.	63	172	60.4	328	340	334	339	334	284	338	347
Mean				278.1	267.7	279.0	275.4	252.2	234.6	259.0	272.8
σ				58.5	64.5	73.7	69.6	69.2	60.1	60.6	69.8
σ_{Mn}				17.6	19.4	22.2	21.0	20.9	18.1	18.3	21.0

Glucose reabsorption (Tm_G) is expressed in mg/min. and is derived as the difference between the rate of filtration of glucose and the rate of urinary excretion of glucose.

* Periods 1-3 represent 3 consecutive control urine collection periods, each of 12 min. duration. Periods 4-8 represent 5 consecutive 12 min. urine collection periods following the intravenous inj. of 20 units crystalline zinc insulin.

Values are not corrected for body surface area.

dwelling needle in the femoral artery. Heparinized blood samples were centrifuged, and plasma was removed within 20 minutes after the sample was obtained. Urine collection periods were terminated by washing out the bladder with physiological saline. Following 3 control urine collection periods, 20 units of crystalline zinc insulin were injected intravenously and 5 successive urine collection periods, each of 12 minutes duration were obtained over the 60-minute period following the injection of insulin. Inulin was determined in diluted urine and in plasma filtrate by the method of Harrison(2). Glucose was determined in diluted urine and plasma filtrate by the spectrophotometric method of Nelson(3).

Results. The intravenous administration of insulin in the present series converted the rising plasma glucose level of the control periods to a progressively decreasing level in the face of continuation of the infusion of glucose at the same or at somewhat augmented rates (Table II). Despite this increase in overall net cellular uptake of glucose, the rate of glucose uptake by the renal tubular cells from the tubular lumen actually decreased (Table I). The decrease in Tm glucose, although small, occurred in all subjects during period 6 and was statistically significant at the 5% level of confidence for period 5 and at the 1% level of confidence for period 6.† That the decrease in Tm_G was not due to a decrease in the filtered load of glucose can be seen in Table II. The rate of filtration of glucose was as great or greater following the injection of insulin than during the control periods. The ratio of filtered glucose to reabsorbed glucose was consistently greater during the post-injection periods than during the control periods. This increase in L/T ratio was due to both a slight increase in the glomerular filtration rate and a slight decrease in the rate of tubular uptake of glucose.

Discussion. The slight, but statistically significant, decrease in Tm_G produced by the administration of insulin to non-diabetic human subjects in the present study is in accord with the findings of Farber *et al.*(4) who reported a reduction in Tm_G when insulin was administered to diabetic subjects. The insulin induced depression in Tm_G is clearly not a phenomenon peculiar to the diabetic individual, as has been suggested, but one which occurs in the non-diabetic subject as well.

† Since each subject served as his own control, tests of significance were based on the differences between the mean of 3 control periods and the period tested computed individually for each subject.

TABLE II. Glomerular Filtration Rate, Plasma Glucose Level, and Rate of Glucose Excretion Following Insulin.*

		Control period			Post-insulin period				
		1	2	3	4	5	6	7	8
Plasma glucose, mg/100 cc	Mn	500.5	545.4	578.3	606.5	599.1	567.8	550.0	522.7
	σ_{Mn}	16.9	15.3	17.4	17.6	19.9	23.5	26.1	32.1
GFR, cc/min.	Mn	95.1	93.3	95.4	97.6	95.6	94.7	99.3	102.5
	σ_{Mn}	5.0	5.6	6.3	6.0	6.3	6.7	6.6	7.3
Filtered glucose, mg/min.	Mn	476.7	509.5	550.1	588.7	571.2	531.0	538.9	538.1
	σ_{Mn}	30.5	33.9	37.2	40.5	41.3	36.4	35.9	41.9
Glucose excretion, mg/min.	Mn	198.5	242.0	271.0	312.4	319.1	296.4	279.9	265.2
	σ_{Mn}	23.3	26.6	26.4	30.2	29.1	26.5	25.2	26.9
Load/T	Mn	1.74	1.99	2.02	2.19	2.34	2.33	2.13	2.01
	σ_{Mn}	.09	.11	.10	.12	.12	.13	.11	.11

Mean values for entire group of 12 subjects.
* See footnote Table I.

In an attempt to reconcile the somewhat paradoxical findings wherein the rate of renal tubular reabsorption of glucose actually decreases during the period when insulin produces a marked increase in overall net cellular uptake of glucose, it will be well to recall that the dynamics of tubular uptake of glucose from the tubular lumen differs in several respects from that of glucose uptake by other cells such as those of skeletal muscle. Glucose is completely reabsorbed by the renal tubule from the filtrate until filtered load of glucose is increased to a point where the reabsorptive mechanism is saturated. The limitation in rate of uptake of glucose from the tubular lumen is most likely due to a limitation in the rate of regeneration of some intracellular substance(5), presumably ATP. On the other hand, glucose is removed from extracellular fluid by muscle at a relatively slower rate. ATP is not a limiting factor in the phosphorylation of glucose by muscle tissue(6), and the rate of cellular uptake is enhanced by insulin. The reabsorption of glucose from the tubular lumen may be considered as a reaction in which glucokinase is abundant relative to the concentration of one of the reactants, ATP; while the cellular uptake of glucose by muscle may be viewed as a reaction in which the enzyme exists in relatively low concentration. Under these circumstances, if the point of action of insulin is on the hexokinase reaction, the hormone would be expected to exert no direct effect on the rate of glucose uptake from the tubular lumen by the tubular cell, and to increase the rate of uptake by muscle cell. The observed reduction in glucose Tm after administration of insulin would not be explained by the above considerations. However, if there exists in the renal tubular cell a mechanism for glucose uptake from peritubular fluid similar to that which exists in muscle tissue, augmentation of this rate of glucose uptake from the blood as a consequence of insulin administration could conceivably reduce the rate of glucose uptake from the tubular lumen by decreasing the amount of available ATP.

Summary. 1. The maximal rate of renal tubular reabsorption of glucose was determined before and after the intravenous administration of 20 units crystalline zinc insulin in a series of 12 non-diabetic male subjects who were free of evidence of cardiovascular or renal disease. Following the administration of insulin, there occurred a slight, but statistically significant, decrease in Tm_G during the period extending from 12 to 48 minutes after injection. 2. The significance of these findings, with respect to the fundamental action of insulin, is discussed.

1. Goldring, W., and Chasis, H., *Hypertension and Hypertensive Disease.* The Commonwealth Fund, New York, 1944.

2. Harrison, H. E., PROC. SOC. EXP. BIOL. AND MED., 1942, v49, 111.

3. Nelson, N., *J. Biol. Chem.,* 1944, v153, 375.

4. Farber, S. J., Berger, E. Y., and Earle, D. P., *J. Clin. Invest.,* 1951, v30, 125.

5. Shannon, J. A., and Fisher, S., *Am. J. Physiol.,* 1938, v122, 765.

6. Slein, M. W., Cori, G. T., and Cori, C. F., *J. Biol. Chem.,* 1950, v186, 763.

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