dication that the essential drive producing gasping phenomena is of chemoreflex origin lies in the nature of these structures. Their extreme ruggedness enables them to activate the central neurones under conditions which render these latter unable to respond to autogenous chemical stimuli. The ability of the carotid body to function during severe anoxia provides the organism with a last line of respiratory defense, Schmidt's ultimum moriens of respiratory control.[6]

[6] Bard, P., *MacLeod's Physiology in Modern Medicine*, C. V. Mosby Co., St. Louis, 1941, p. 593.

I am indebted to Dr. R. W. Gerard of the Department of Physiology, University of Chicago, and D. B. Gordon for assistance in the preparation of this report.

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Mutual Depression of Reabsorption and Excretory Maxima in Renal Tubules.

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It is generally understood that the capacity of the renal tubule cells to transport a substance in one direction is not influenced by the simultaneous transport of another substance in the opposite direction. Thus Smith *et al.*[1] report that simultaneous determinations of the maximal tubular reabsorption of glucose (Tm_G) and the maximal tubular excretion of *p*-aminohippuric acid (Tm_{PAH}) yield the same values as separate determinations, and elsewhere Smith[2] illustrates a set of simultaneous measurements of these 2 functions. This is also presumably true for the dog.[3,4] However, when individual experiments are examined, there is, in some instances, evidence of depression of Tm_G by PAH and of Tm_{PAH} by glucose. We have therefore reexamined this question of mutual depression of the transport of glucose and PAH in the dog.

Experimental. Tm_G and Tm_{PAH} were determined in 3 trained, unanesthetized female dogs ranging in body weight from 17 to 24 kg. Tm_G alone was determined during 3 consecutive urine collection periods of approximately 10 minutes each. After a priming dose of PAH, this substance was added to the infusion fluid and Tm_{PAH} and Tm_G were determined simultaneously during 3 additional periods. On another day this procedure was repeated in the same dog but the substances were reversed, *i.e.*, Tm_{PAH} alone was measured first. The creatinine clearance was taken as a measure of the glomerular filtration rate. The dogs were hydrated with 50 cc of water per kg of body weight by stomach tube 45 minutes before U_O. Plasma levels of creatinine, glucose and PAH were obtained by suitable priming injections into the jugular vein 10 minutes before U_O and maintained by constant intravenous infusion into a leg vein by a gravity mercury-drip pressure apparatus. In all experiments the load/Tm ratio for both glucose and PAH was well above 1.5. Urine was collected by an indwelling bladder catheter and blood was withdrawn periodically from the femoral artery through a 19-gauge retention needle.

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[1] Smith, H. W., Goldring, W., Chasis, H., Ranges, H. A., and Bradley, S. E., *J. Mt. Sinai Hosp.*, 1943, **10**, 59.

[2] Smith, H. W., *Porter Lectures*, Series 9, 1939, Univ. Extension Division, Univ. of Kansas, Lawrence, Kansas. Revised 1943.

[3] Selkurt, E. E., *Am. J. Physiol.*, 1944, **142**, 182.

[4] Eiler, J. J., Althausen, T. L., and Stockholm, M., *Am. J. Physiol.*, 1944, **140**, 699.

TABLE 1.
Mutual Depression of Renal Tubular Maxima (Tm's) of Glucose and *p*-Aminohippuric Acid (PAH) in the Dog.

Dog	Date of exp.	Glucose Tm* Alone mg/min	Glucose Tm* During PAH infusion mg/min	Tm_G with PAH† / Tm_G alone	Avg Tm_G with PAH‡ / Avg Tm_G alone	PAH Tm* Alone mg/min	PAH Tm* During glucose infusion mg/min	Tm_{PAH} with G† / Tm_{PAH} alone	Avg Tm_{PAH} with G‡ / Avg Tm_{PAH} alone
R	7/24/46	250	228	0.91		—	13.6	—	
	29	238	219	0.92		—	16.6	—	
	8/ 1	—	211	—		19.8	16.7	0.84	
	5	—	181	—		23.6	17.7	0.75	
	Avg	244	210	0.92	0.86	21.7	16.2	0.80	0.75
	σ	11.8	22.8			2.3	2.1		
	Avg-2σ	220				17.2			
A	7/26/46	224	192	0.86		—	13.8	—	
	30	212	186	0.88		—	13.1	—	
	8/ 2	—	209	—		18.3	15.8	0.86	
	7	—	188	—		16.1	13.9	0.86	
	Avg	218	194	0.87	0.89	17.2	14.2	0.86	0.83
	σ	11.7	16.5			1.4	1.7		
	Avg-2σ	195				14.4			
B	8/23/46	172	171	0.99		—	9.9	—	
	9/12	155	135	0.87		—	12.4	—	
	7/19	—	128	—		16.1	12.7	0.79	
	Avg	164	145	0.93	0.88	16.1	11.7	0.79	0.73
	σ	14.9	20.4			0.83	1.6		
	Avg-2σ	134				14.4			
Avg				0.91	0.88			0.82	0.77

* Each value is an average of 3 consecutive 10-minute urine collection periods.
† Ratio based on Tm's studied on same day.
‡ Ratio based on average of all Tm's studied.
σ = Standard deviation from mean.

Plasma proteins were precipitated by cadmium sulfate and NaOH, and creatinine and PAH were analyzed as described by Smith *et al.*,[5] while glucose was determined by Shannon's modification[6] of the Folin method. All colorimetric determinations were made in duplicate and read on an Evelyn photoelectric colorimeter.

Tm_{PAH} in mg/min. was calculated by subtracting the amount filtered per minute (0.83 × plasma concentration in mg/cc × filtration rate in cc/min.) from the total amount excreted per minute (urine concentration in mg/cc × urine flow in cc/min.) Tm_G in mg/min. was calculated by subtracting the amount excreted per minute (urine concentration in mg/cc × urine flow in cc/min.) from the amount filtered per minute (plasma concentration in mg/cc × filtration rate in cc/min.)

Results. The values for Tm_G and Tm_{PAH} determined alone or simultaneously are given in Table I where each value represents the average of 3 consecutive 10-minute urine collection periods. The ratios:

$$\frac{Tm_G \text{ with PAH}}{Tm_G \text{ alone}} \quad \text{and} \quad \frac{Tm_{PAH} \text{ with glucose}}{Tm_{PAH} \text{ alone}}$$

are also presented when values in both denominator and numerator were determined consecutively on the same day. Along with these ratios are included ratios whose numerator and denominator represent the overall average of all values obtained in each category, *i.e.*, on different days as well as on the same day.

It is evident that the tubular transport mechanisms for glucose and PAH in the dog are not mutually independent, as previously reported, because saturation of the transport mechanism by one substance depresses the transport of the other. The depression is of small magnitude and might be dismissed as not significant. However, some depression occurs consistently in each of the 3 dogs studied, and in 2 out of 3 dogs, the mean of all Tm_G values measured simultaneously with Tm_{PAH} fall outside the limit of 2 σ† from the mean of Tm_G measured alone. Likewise, in 2 out of 3 dogs, the mean of Tm_{PAH} measured during glucose saturation falls outside the limit of 2 σ from the mean of Tm_{PAH} measured alone, and is on the borderline of this limit in the third dog. When all data on each dog are averaged, the percentile decrease in Tm_G during PAH infusion is practically the same in each of the 3 dogs. The percentile decrease in Tm_{PAH} during glucose infusion is greater in 2 dogs than in the third. In every case, the percentile depression of Tm_{PAH} is greater than the percentile depression of Tm_G, indicating a more marked effect of glucose on PAH transport than of PAH on glucose transport. Averaging the data of the 3 dogs, Tm_G is depressed 12%; Tm_{PAH}, 23%; *i.e.*, the effect of glucose on PAH transport is nearly twice as great as the effect of PAH on glucose transport.

Selkurt[3] studied Tm_G alone and simultaneously with Tm_{PAH} in 4 dogs, and reported ratios of Tm_G with PAH/Tm_G alone of 0.92, 0.98, 0.88 and 1.06, respectively, with an overall average of 0.96. Therefore, he concluded that PAH did not influence Tm_G. Eiler, Althausen, and Stockholm[4] have reported data on one dog which indicate a slight mutual depression of Tm_G (11%) and $Tm_{diodrast}$ (18%), although they make no particular mention of it. In another dog, $Tm_{diodrast}$ was decreased 12% by glucose. As mentioned above, Smith *et al.*[1,2] have found no mutual interference of glucose and diodrast transport in normal man, and therefore state that tubular maxima of these substances may be studied simultaneously. However, they do not particularly recommend this procedure, "since it offers opportunities for unanticipated complications" (p. 92). In this regard, Klopp, Young and Taylor[7] report a decrease in Tm_{PAH} with glucose in 3 normal individuals, and an increase in Tm_G with PAH in 4 other individuals.

The mutual depression of Tm_G and Tm_{PAH} obtained in the present study may be due

[5] Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. Clin. Invest.*, 1945, **24**, 388.

[6] Shannon, J. A., Farber, S., and Troast, L., *Am. J. Physiol.*, 1941, **133**, 752.

† σ = Standard deviation from the mean.

[7] Klopp, C., Young, N. F., and Taylor, H. C., Jr., *J. Clin. Invest.*, 1945, **24**, 117.

to a deflection of available energy necessary for transport[3] rather than to competition for a specific cellular constituent or "B substance" involved in the transfer of solutes across renal tubule cells.[8] It seems unlikely that the same B substance would be used in the transport of solutes in opposite directions within the cell, when solutes traveling in the same direction may have a different B substance, *e.g.*, glucose and glycine.[9] Assuming that the B substance of PAH is different from that of glucose, the cause of mutual interference must be sought in some portion of the transport mechanism common to each. As supporting evidence, Selkurt[3] has pointed out the fact that both Tm_G and $Tm_{diodrast}$ can be reduced by phlorizin.[2] In this regard, it seems more reasonable to suggest that the energy necessary for combination of either glucose or PAH with its B substance, that energy for the release from this substance at the terminus of transport, or that energy required for transport across the cell is not available in sufficient quantity to satisfy the simultaneous demands of PAH and glucose. In other words, this available energy may be the limiting factor in the capacity of the tubules when they are carrying these solutes in opposite directions simultaneously.

Conclusion. In the dog, the maximum renal tubular reabsorption of glucose (Tm_G) and the maximum tubular excretion of *p*-aminohippuric acid (Tm_{PAH}) are mutually depressed when determined simultaneously. The percentile depression of PAH by glucose is twice as great as the depression of glucose by PAH. This depression is attributed to competition for available energy rather than to competition for a common cellular constituent (B substance) involved in tubular reabsorption and excretion.

[8] Shannon, J. A., *Physiol. Rev.*, 1939, **19**, 63.

[9] Pitts, R. F., *Am. J. Physiol.*, 1943, **140**, 156.

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Improved Fixation for Histological Demonstration of Glycogen and Comparison with Chemical Determination in Liver.*

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Previous attempts to demonstrate a quantitative correlation between the amount of glycogen present in liver as determined by chemical procedures and as revealed by histological examination have been unsuccessful.[1,2] It is the purpose of the present paper to present data showing that under certain conditions such a correlation can be demonstrated. The increasing number of histochemical studies of tissues employing chemical technics on the one hand and histological technics on the other makes such a comparative study desirable.

This study was undertaken after it had been observed that glycogen can be preserved uniformly throughout histological blocks by placing pieces of organs in ice-cold fixative, instead of fixing blocks at room temperature as had been done heretofore.

Material and Methods. In order to prepare animals with variable concentrations of liver glycogen, a method originally suggested by Higgins, Berkson and Flock[3] was employed. For 5 days before sacrifice, 20 male

* This work was done in part under a grant from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council.

[1] Grafflin, A. L., Marble, A., and Smith, R. M., *Anat. Rec.*, 1941, **81**, 495.

[2] Eger, W., *Virchow's Arch.*, 1942, **309**, 607.

[3] Higgins, G. M., Berkson, G., and Flock, E., *Am. J. Physiol.*, 1932, **102**, 673; *ibid.*, 1933, **105**, 177.