

and cut at the point of origin of the spermatic duct. In some cases the removal of the testes was done in 2 stages.

The experimental animals were examined after 2, 3 or more months, and showed regeneration of the testes in about 50% of the cases. Bilateral regeneration was almost always observed, and often one of the testes was much smaller than the other. In many cases there was regeneration 2 months after the operation. Histological sections showed the typical structure of the testes, with spermatocytes in all stages of development (Figure 1) and even tufts of sperm (Figure 2) in some cases. The great development of the fat bodies in the animals with regenerated testes should be noted. In all cases the fat bodies were in direct communication with the regenerated testes.

The origin of the regenerated testes is not known as yet. The most obvious hypothesis is that they are derived from somatic cells of the sperm duct which have de-

differentiated and then redifferentiated. They are clearly not derived from fragments of testes accidentally left after the operations. A more extensive paper is in preparation.

*Riassunto.* Dopo ablazione totale dei testicoli in *Disco-glossus pictus* si ha rigenerazione. Nei testicoli rigenerati si riscontrano spermatociti in tutti gli stadi di sviluppo e talvolta anche spermi. È avanzata l'ipotesi che la rigenerazione avvenga a partire da cellule somatiche degli spermidutti.

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### The Distribution of Glucose and Methyl-Glucose Between the Liver and Plasma in Normal and Insulin Injected Rats

It appears to be a well established fact that in vivo glucose enters the liver cells rapidly and is contained therein at a concentration slightly higher than in the blood plasma<sup>1,2</sup>. This calculation involves the assumption that glucose in the liver is distributed uniformly in the total volume of tissue water. However, if some part of the cell water is not accessible to glucose its actual concentration in other parts of the cell water has to be higher than estimated. Insulin was shown to increase the apparent glucose space of the liver, i.e. the ratio of the concentrations of glucose in liver tissue and plasma<sup>3</sup>. The validity of this finding was challenged<sup>4</sup> by pointing out that the determination of the glucose space of the liver in the absence of a dynamic steady state of plasma glucose may lead to a biased estimate<sup>4</sup>.

The aims of the present study are: (1) to establish the distribution space of an unmetabolizable glucose analogue, 3-methyl-glucose (MEG) in liver water; (2) to calculate the average glucose concentration in the 'available' water of the liver, assuming that MEG and glucose enter the same volume of liver water; and (3) to establish whether insulin has any effect on the distribution space of MEG and/or the apparent distribution space of glucose in the liver of animals which are in or close to a dynamic steady state with respect to their glucose and MEG levels in the plasma.

*Methods.* Experiments were carried out on fasted male rats under Nembutal anaesthesia. A priming dose of 0.25  $\mu$ C (18  $\mu$ g) of <sup>14</sup>C-labelled MEG injected into the jugular vein was followed by a 0.004  $\mu$ C/min (0.012  $\mu$ g/min) infusion of MEG to keep the concentration of MEG constant in the plasma. After 60 or 120 min, 0.4 ml blood was taken from the aorta and a quick-frozen<sup>5</sup> sample of liver was removed. Another liver sample was weighed and then dried at 110 °C for 24 h. One gastrocnemius was analysed for <sup>14</sup>C activity and the other dried as above. 5 rats received i.p. injections of crystalline insulin (Toronto): 1 U at the beginning and 1 U at the mid point of the 60 min infusion of MEG.

Glucose and MEG were extracted from liver and muscle as described by MORGAN<sup>6</sup>. Glucose was determined enzymatically<sup>7</sup> and MEG as the radioactivity present in the extracts. Total tissue water (ml/g) was calculated as the difference between the wet and dry weight of the sample, plasma was assumed to contain 0.94 ml H<sub>2</sub>O/g, the ratio of the concentrations in total tissue water and plasma water will be referred to as the calculated distribution space of MEG and/or glucose. Since MEG is not metabolized, the distribution space of MEG was taken as an estimate of the actual volume of water in which MEG and presumably glucose are distributed.

*Results and discussion.* The results are summarized in the Table. It appears that MEG equilibrates with only about 85% of total tissue water in the liver. This calculated distribution volume was not increased significantly by prolonging the infusion of MEG from 1–2 h, the equilibrium was 'complete' after 1 h. As insulin did not cause an increase in the distribution volume of MEG, it appeared that no part of liver water inaccessible to MEG had been made accessible to it by insulin. Our results are in essential agreement with those of BERTHET et al.<sup>8</sup>, who found that <sup>14</sup>C-glucose equilibrates with about 72% of the total water of incubated liver slices, and this % is not altered by a concentration of insulin active in other respects. CSAKY and GLENN<sup>9</sup> found that after a single injection, unlabelled MEG was dissolved in 100% of total liver water in nephrectomized rats. The slight discrepancy

<sup>1</sup> K. F. GEY, *Biochem. J.* **64**, 145 (1956).

<sup>2</sup> G. HETENYI JR. and G. S. ARBUS, *J. gen. Physiol.* **45**, 1049 (1962).

<sup>3</sup> R. STEELE, *Ergebn. Physiol* **57**, 91 (1966).

<sup>4</sup> D. S. RIGGS, *The Mathematical Approach to Physiological Problems* (William and Wilkins Co., Baltimore 1963).

<sup>5</sup> A. WOLLENBERGER, O. RISTAU and G. SCHOFFA, *Pflügers Arch. ges. Physiol.* **270**, 399 (1960).

<sup>6</sup> H. E. MORGAN, P. J. RANDLE and D. M. REGEN, *Biochem. J.* **73**, 573 (1959).

<sup>7</sup> A. S. G. HUGGET and D. A. NIXON, *Biochem. J.* **66**, 12P (1957).

<sup>8</sup> P. BERTHET, P. JACQUES, H. G. HERS and C. DE DUVE, *Biochim. biophys. Acta* **20**, 190 (1956).

<sup>9</sup> T. Z. CSAKY and J. E. GLENN, *Proc. Soc. exp. Biol. Med.* **98**, 400 (1958).

Distribution of 3-methyl-glucose (MEG) and glucose between liver, muscle and plasma water in anaesthetized rats after an infusion of MEG-<sup>14</sup>C

Length of MEG infusion (min)	No. of experiments	Insulin	Average glucose concentration in plasma-H <sub>2</sub> O mg/100 ml	Distribution space of MEG, ml/g		Ratio of glucose concentration in liver-H <sub>2</sub> O/plasma-H <sub>2</sub> O <sup>b</sup>	Ratio of glucose concentration in 'available' liver-H <sub>2</sub> O/plasma-H <sub>2</sub> O <sup>c</sup>
				Liver <sup>a</sup>	Gastrocnemius		
60	5	-	82.9	0.787 ± 0.047	0.220 ± 0.019	1.395 ± 0.154	1.776 ± 0.196
120	4	-	76.2	0.919 ± 0.059	0.239 ± 0.014	1.506 ± 0.067	1.655 ± 0.115
60	5	+	27.7	0.864 ± 0.041	0.666 ± 0.058	2.258 ± 0.347	2.632 ± 0.432

Average values ± standard error of means are shown. <sup>a</sup> The overall average of the 2 groups not treated with insulin is 0.846 ± 0.042.

<sup>b</sup> The overall average of the 2 groups not treated with insulin corresponds to an average ratio of 1.13 for mg glucose in g liver/mg glucose in ml plasma. <sup>c</sup> Equals the calculated apparent distribution volume of glucose/calculated distribution volume of MEG. The overall average of the groups not treated with insulin is 1.722 ± 0.112.

between their results and ours is due either to differences in the chemical methods used, or to the divergence between injection and steady state infusion methods in estimating distribution spaces<sup>4</sup>. In agreement with earlier investigators an increased distribution space of MEG was found in the gastrocnemius of rats treated with insulin<sup>10</sup>.

The calculated apparent distribution space of glucose in the liver was larger than the MEG space. Assuming that the same volume of water is available to dissolve both sugars, the average concentration of glucose was 1.72 ± 0.11 times as high in this water as in plasma water. Since, however, in rats about 30% of the total liver water<sup>11</sup>, and thus 36% of the distribution volume of MEG, is extracellular, the true concentration ratio of glucose between the glucose-containing compartment of the liver cell and extracellular water is likely to be about 2.0. In insulin induced hypoglycaemia the ratio of the concentrations of glucose in the MEG-space and plasma water was 2.63 ± 0.43, the estimated true intracellular glucose concentration being 3.3 times as high as in the plasma. Since insulin did not cause an accumulation of MEG in the liver, its effect on the calculated distribution space of glucose is not likely to be due to an alteration of permeability of the liver cell membrane. It appears that this increase is the result of the circumstances that the outward movement of glucose is not facile enough to pre-

vent the existence of a considerable concentration gradient when net glucose flow out of the liver is going on<sup>8</sup>, especially at an increased rate as in hypoglycaemia<sup>12</sup>.

**Zusammenfassung.** Es wurde gefunden, dass 3-Methyl-Glukose (MEG) sich in 85% der gesamten Gewebeflüssigkeit der Rattenleber löst. Ist Glukose in gleicher Plasmamenge enthalten, so ist die berechnete Konzentration in der Leberzellflüssigkeit doppelt so hoch. Bei Insulinhypoglykämie stieg dieses Verhältnis weiter an, ohne aber eine Veränderung im Verteilungsvolumen von MEG zu ergeben. Die Leberzellmembran scheint die Penetration der Glukose aus der Zelle zu verzögern.

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<sup>10</sup> H. T. NARAHARA and P. ÖZAND, J. biol. Chem. 238, 40 (1963).

<sup>11</sup> J. F. MANERY and A. B. HASTINGS, J. biol. Chem. 127, 657 (1939).

<sup>12</sup> The financial support of The Medical Research Council of Canada and the Banting Foundation as well as the skilled technical help of Mrs. S. WATERFIELD is gratefully acknowledged.

## Similar Effects of Arginine-Vasopressin and Arginine-Vasotocin on Permeability of Toad Skin

Neurohypophysial peptides have been generally assayed on test objects which detect smooth muscle activity (uterus, blood pressure, and mammary gland) or fluid reabsorption (rat anti-diuresis and amphibian bladder), and the 2 types of response have been sometimes thought to be related to augmented sodium transport and to increased water permeability. While vasopressor activity has seemed to be associated with basicity of the amino acid in position 8<sup>1</sup>, and oxytocic activity has seemed to depend on iso-leucine in position 3 and the integrity of the peptide ring<sup>2</sup>, the responses of various tissues to

natural or synthetic hormones have seldom been in agreement. BOURGUET and MAETZ<sup>3</sup> have shown for several peptides that there is a lack of correlation between natriuretic and hydrosmotic activity. It was therefore of interest to examine the effect of various peptides

<sup>1</sup> P. G. KATSOYANNIS and V. DU VIGNEAU, Archs Biochem. Biophys. 78, 555 (1958).

<sup>2</sup> J. RUDINGER and K. Jošt, in *Oxytocin, Vasopressin and Their Structural Analogues* (Ed., J. RUDINGER; Pergamon Press, London 1964).

<sup>3</sup> J. BOURGUET and J. MAETZ, Biochim. biophys. Acta 52, 552 (1961).