

in hormonal state induced by the pregnancy could be considered an important factor for variations in tissue glycogen. The observed variations in glycogen concentration of the uterus support the suggestion that an active transport of hepatic and cardiac glycogen to the uterus exists during late pregnancy.

Résumé. Ce travail décrit l'évolution du contenu en glycogène de l'utérus, du foie et du cœur chez des rattes en fin de gestation, au moment de la parturition et en post-partum. Le glycogène de l'utérus augmente de façon très significative du 18e jour de gestation jusqu'au moment de la parturition. Au contraire le glycogène

hépatique diminue du 18e au 21e jour de gestation. Le contenu en glycogène du cœur décroît brutalement du 20e jour de gestation à 0 h post-partum. Dès 24 h post-partum les réserves en glycogène de chacun des organes amorcent un retour vers les valeurs témoins. Les résultats suggèrent qu'il existe un déplacement du glycogène hépatique vers l'utérus pendant la dernière partie de la gestation.

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A Radioimmunoassay of Vasopressin. A Note on Pituitary Vasopressin Content in Brattleboro Rats

A recently developed radioimmunoassay of vasopressin was tested for its sensitivity and reliability by determining the amount of vasopressin in posterior pituitaries of rats with hypothalamic hereditary diabetes insipidus (D. I.) (Brattleboro strain). Posterior pituitary vasopressin content of heterozygous D.I. rats has been reported¹, but little data on pituitary vasopressin content of homozygous D.I. rats exist. Therefore, the amount of vasopressin in posterior pituitaries of homozygous and heterozygous rats of the Brattleboro strain was assessed by both radioimmunoassay and bioassay.

Antibodies against synthetic arginine-8-vasopressin (AVP; 300 IU/mg, Ferring) were produced as described by SKOWSKY and FISHER². AVP was coupled to thyroglobulin by ethylcarbodiimide and the product was emulsified in Freund's complete adjuvant and injected i.m. into young adult New Zealand rabbits (1.5–3.0 kg).

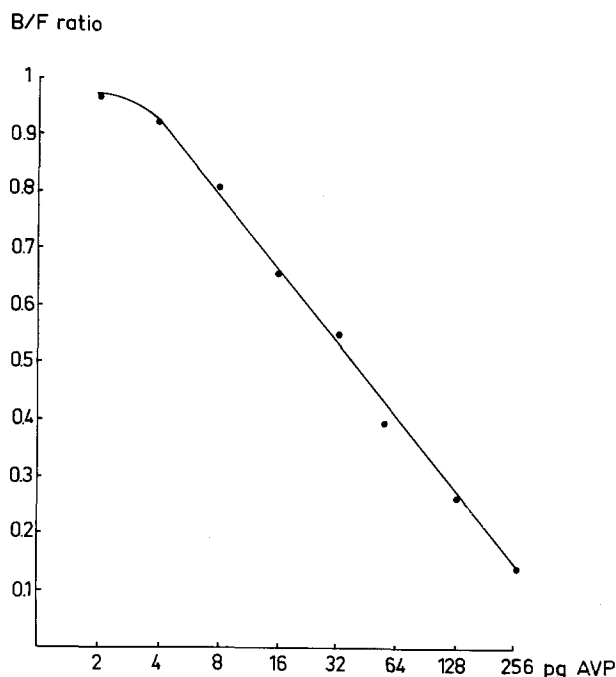
An initial dose of antigen, corresponding to 0.4 mg AVP was given, followed by a booster with half this amount of antigen every 3 weeks. The first antiserum was collected 18 weeks after the initial injection.

AVP was labelled with Na¹²⁵I (Amersham) using chloramine T as oxydant. The reaction was stopped by addition of human serum. Separation of ¹²⁵I-AVP, from labelled albumin and unlabelled AVP, was accomplished by passage through a Sephadex G 25 fine column (1.0 × 10.0 cm) equilibrated with 0.2 M acetic acid. This tracer with a specific activity of 200 μ Ci/ μ g was stored in small portions at –20°C. A Veronal buffer (pH 8.0) was used as diluent³.

Standard amounts of AVP ranging from 1 pg to 128 pg were used for the calibration curve (Figure), which included antibody blank and diluent blank. The final dilution of the antibody was 1:60,000. The total volume was 110 μ l/tube. The incubation time was 48 h at 4°C. Ammonium sulphate was used for the separation of bound and free ¹²⁵I-AVP. The detection limit in buffer was 2 pg AVP. Similar standard curves for AVP were obtained, ranging from 2 to 128 pg over a number of assays, and also the same detection threshold of 1–3 pg was found, indicating a high degree of reproducibility. Concerning cross reactivity with related peptides, the detection thresholds of oxytocin and of lysine-8-vasopressin (LVP) were ca. 60 pg. Standard curves with these peptides did not run parallel to the AVP curve.

The pressor activity of the posterior pituitary extracts was estimated by the blood pressure bioassay in anesthetized rats pretreated with phenoxybenzamine (10 mg/kg) as described by DEKANSKI⁴.

Homozygous and heterozygous male rats of the Brattleboro strain, bred under SPF conditions (TNO, Zeist) were killed by decapitation. The pituitaries were removed from the sella, the posterior lobes separated from the anterior lobes, weighed, homogenized in 2.1 ml diluent buffer and stored at –20°C until testing. Aliquots of pituitary homogenates of homozygous and heterozygous rats were tested for pressor activity in a 2 × 2 bioassay. In 3 posterior pituitary homogenates of homozygous D.I.



Standard curve of arginine-8-vasopressin (AVP) showing inhibition of binding of ¹²⁵I-AVP by antibody at a final dilution of 1:60,000 in the presence of serial dilutions of unlabelled AVP. The bound/free ratio of ¹²⁵I-AVP is plotted against the concentration of standard AVP.

¹ A. M. MOSES and M. MILLER, *Endocrinology* 85, 34 (1970).

² W. R. SKOWSKY and D. A. FISHER, *J. Lab. clin. Med.* 80, 134 (1973).

³ G. L. ROBERTSON, J. ROTH, C. BEARDWELL, L. A. KLEIN, M. J. PETERSON and PH. GORDEN, in *Methods in Investigative and Diagnostic Endocrinology* (Eds. S. A. BERSON and R. S. YALOW; North-Holland Publ. Comp., Amsterdam/London, 1973), Vol. IIA, part II, p. 656.

⁴ J. DEKANSKI, *Brit. J. Pharmac.* 7, 567 (1952).

Posterior pituitary vasopressin content in rats homozygous and heterozygous for hereditary diabetes insipidus as measured by radio-immunoassay and bioassay

	Body weight (g)	Posterior pituitary weight (mg)	Vasopressin (mU/posterior pituitary)		Correlation RIA/bioassay (r)
			RIA	Bioassay	
Homozygous	243 \pm 6	1.24 \pm 0.07	8.2 \pm 2.0	11.5 \pm 2.8	0.83 ($p < 0.05$) (7)
Heterozygous	283 \pm 7	0.84 \pm 0.03	163.0 \pm 22.7	169.0 \pm 27.4	0.97 ($p < 0.01$) (7)

Values are mean \pm S.E.M. Numbers of animals in parentheses.

rats, the vasopressin content was very low as measured by radio-immunoassay. In these samples, a 2×2 pressor assay was not feasible and a 2×1 assay was performed. Immunoassay of aliquots was performed in triple on 1:25 dilutions of pituitary homogenates of homozygous rats and on 1:250 dilutions of those of heterozygous animals.

Although body weight of the homozygous rats was less than that of the heterozygous ones, the posterior pituitary weight was significantly higher in the former group (Table) confirming the data of ARIMURA, SAWANO, REDDING & SCHALLY⁵.

Vasopressin content of pituitaries of homozygous rats was extremely low compared to that of heterozygous animals as measured by bioassay as well as immunoassay (Table). A close correlation was found when pituitary vasopressin content by immunoassay (Y) was plotted against this content by bioassay (X). For heterozygous animals $r = 0.97$ ($p < 0.01$) ($Y = 1.2 X - 29.9$) and for homozygous animals $r = 0.83$ ($p < 0.05$) ($Y = 1.2 X + 2.0$). These results are in good agreement with those of MOSES and MILLER¹ and of VALTIN et al.⁶.

Oxytocin content of the homozygous D.I. rats is greatly reduced as compared to that of heterozygous animals⁶. Moreover this oxytocin content contributes only to a small degree ($< 2\%$) to the vasopressin-like activity in the posterior pituitary of homozygous D.I. rats as determined by blood pressure bioassay⁷. Additionally the dilution curves obtained with aliquots of pituitary homogenates in the radio-immunoassay were parallel to the AVP standard curves, in contrast with those obtained with oxytocin. Thus it is unlikely that oxytocin contributes significantly to the vasopressin-like activity that is found in posterior pituitaries of Brattleboro rats.

In the homozygous D.I. rats of the Brattleboro strain, posterior pituitary vasopressin-like activity is minimal as compared to that of heterozygous animals. The radio-

immunoassay of AVP described, represents a sensitive method for the quantitative measurement of biologically active vasopressin^{8,9}.

Zusammenfassung. Die Empfindlichkeit und Zuverlässigkeit eines neu entwickelten Radioimmunotests für Vasopressin wurde durch Messungen des Vasopressinspiegels im Hypophysenhinterlappen von homozygoten und heterozygoten Ratten für angeborenen Diabetes insipidus geprüft und eine gute Korrelation zwischen dem biologisch und radioimmunologisch gemessenen Vasopressingehalt der Hypophyse nachgewiesen.

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⁵ A. ARIMURA, S. SAWANO, T. W. REDDING and A. V. SCHALLY, *Neuroendocrinology* 3, 187 (1968).

⁶ H. VALTIN, W. H. SAWYER and H. S. SOKOL, *Endocrinology* 77, 701 (1965).

⁷ H. B. VAN DIJKE, K. ADAMSON and S. L. ENGEL, *Recent Prog. Horm. Res.* 9, 1 (1955).

⁸ The skilful technical assistance of Miss M. A. COLENBRANDER is gratefully acknowledged.

⁹ These investigations were partly subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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The Effect of Theophylline on Oxytocin Induced Contractions in the Chronically Catheterized Pregnant Rabbit

The sensitivity of the pregnant rabbit myometrium to intra-aortic oxytocin infusion increases as the circulating plasma progesterone concentration falls^{1,2}. SMITH, ABEL and NATHANIELSZ² have described an experimental preparation in which catheters are introduced into the femoral artery and vein of a 21 day pregnant rabbit under nembutal anaesthesia. The catheters are advanced cranially until their tips lie in the aorta and vena cava, above the level of the ovarian blood vessels. If the animals

are infused continuously with saline they deliver live litters approximately 185 h after operation, whereas the infusion of prostaglandin F_{2α} (PGF_{2α}) at doses of

¹ P. W. NATHANIELSZ, M. ABEL and G. W. SMITH, *Proceedings of the Sir Joseph Barcroft Centenary Symposium* (Ed. R. S. COMLINE; Cambridge University Press 1973), p. 594.

² G. W. SMITH, M. ABEL and P. W. NATHANIELSZ, *Prostaglandins* 3, 525 (1973).