

In previous experiments<sup>7</sup>, it was found that the degree of regression of the female Müllerian ducts could be related to the quantity of testicular tissue developed in the implants at autopsy. A subliminal amount of testicular tissue caused no regression, a liminal amount caused an incomplete regression, and a minimal amount was required to produce a complete regression.

A 6-day-old testis, implanted on the 4th day of incubation, usually caused a complete regression. In order to be able to assess the functional activity of the implanted testicular tissue, it was necessary to implant parts of 5–8 days old testes, which would cause varying degrees of regression. The preliminary results of these experiments are listed in Table II. As judged from the relation between the degree of regression of the host's Müllerian ducts and the quantity of testicular tissue developed in the implant expressed in PW, no difference in functional activity is found between intracoelomic and intraventricular implants. This observation provides sufficient evidence that the implants are functionally equivalent. Moreover, it is interesting to note that so far no influence is noticed of the different distance of the two sites of implantation from the target organ.

The experiments reported here illustrate that the intraventricular implantation is a valuable complement to other methods of implantation. Apart from its specific usefulness for developmental embryology, it may be of interest to workers in various fields of research, such as comparative endocrinology, neurology and cancer research<sup>8</sup>.

*Zusammenfassung.* Methode zur Implantation mehrerer Gewebestückchen (Hypophyse und Testes) in den IV. Ventrikel 4–5 Bruttage alter Hühnerembryonen, deren funktionelle Vollwertigkeit demonstriert wird.

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### The Wash-Out of Intraarterially Injected Krypton<sup>85</sup> from the Intestine of the Cat

Techniques have recently been developed for quantitatively measuring blood flow and flow distribution in different tissues by recording the disappearance of radioactive inert gases injected intraarterially<sup>1,2</sup>. These methods are based on the theoretical considerations originally developed by KETY<sup>3</sup>. The tissue clearance technique seems a priori to be a suitable method for studying blood flow distribution within the intestinal wall since it contains anatomically well defined parts, such as the muscularis and the villi. In this preliminary report, experiments are described in which the above-mentioned technique has been applied to the intestine of the cat.

*Methods.* The experiments were performed on cats deprived of food for at least 24 h and anaesthetized with chloralose (50–70 mg/kg). Venous outflow from a section of the jejunum weighing 20–50 g was recorded by means of a drop recorder unit operating an ordinate writer. Arterial inflow pressure was monitored from the left femoral artery by a mercury manometer. Atropin was given (1 mg/kg) and the splanchnic nerves were cut bilaterally. Intravenous infusions of isopropylnoradrenalin to produce intestinal vasodilatation were made via a catheter in the left femoral vein.

0.4–0.8 ml (0.3–0.6 mC) of a saline solution containing the radioactive isotope Kr<sup>85</sup> was given as a single injection, lasting 5–10 sec, through a small branch of the superior mesenteric artery. The  $\gamma$ -radiation was recorded by an external scintillation detector collimated in such a way that only radiation from the intestine was registered. The activity was recorded with a spectrometer and a linear ratemeter operating a Rikadenki recorder. In most experiments  $\beta$ -activity was also recorded by means of a Geiger-Müller tube (Philips No. 18509) placed either in the lumen of the intestine or against the outside of the gut in such a way that only activity from the intestinal wall

was registered. The wash-out curve minus background was plotted semilogarithmically and the multiexponential curve was analysed by successively subtracting exponentials as originally proposed by DOBSON and WARNER<sup>2,4</sup>.

*Results.* The Figure illustrates the data from a representative experiment. The upper panel shows a semilogarithmic plot of the Kr<sup>85</sup> disappearance curve as monitored by the external gamma probe (heavy curved line). A continuous record of arterial blood pressure and venous outflow from the jejunum was simultaneously obtained (lower panel). Blood flow and blood pressure remained throughout constant except for a small, transient flow increase when injecting the isotope (time zero). The decay of radioactivity is multiexponential and is the sum of 4 monoexponential components indicated by the thin straight lines of the figure (components I–IV). Since the very slow component (IV) evidently represents blood flow of a tissue outside the intestinal wall, this component was constructed from the decay of the  $\gamma$ -radiation after the  $\beta$ -radiation, registered by a G-M tube in the lumen of the intestine, had returned to background level, indicating that no radioactivity remained in the intestinal wall (see discussion). The table in the upper right part of the Figure gives some pertinent data obtained from the wash-out curve. The Table summarizes 11 experimental runs.

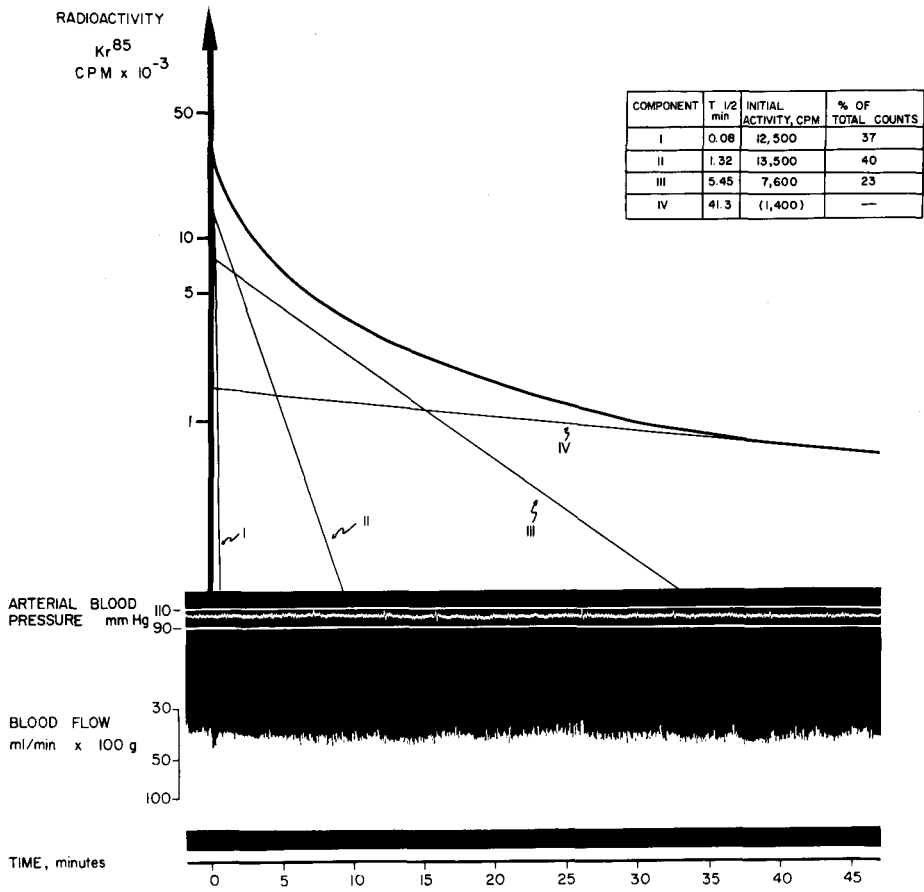
*Discussion.* The experiments reported in this study indicate that the wash-out curve of intraarterially injected Kr<sup>85</sup>, recorded from the intestine of the cat, can be

<sup>1</sup> J. A. HERD, M. HOLLENBERG, G. D. THORBURN, H. H. KOPALD, and A. C. BARGER, *Am. J. Physiol.* 203, 122 (1962). – D. H. INGVAR and N. A. LASSEN, *Acta physiol. scand.* 54, 325 (1962).

<sup>2</sup> G. D. THORBURN, H. H. KOPALD, J. A. HERD, M. HOLLENBERG, C. C. C. O'MORCHOE, and A. C. BARGER, *Circ. Res.* 13, 290 (1963).

<sup>3</sup> S. S. KETY, *Pharmacol. Rev.* 3, 1 (1951).

<sup>4</sup> E. L. DOBSON and G. F. WARNER, *Am. J. Physiol.* 189, 269 (1957).



Cat 2.4 kg. Upper panel: the disappearance of intraarterially injected  $Kr^{85}$  from the jejunum (heavy curved line). Graphic representation of the resultant exponentials is shown by thinner straight lines (see text). Pertinent data obtained from the curve is given in the accompanying table. Lower panel: continuous record of arterial inflow pressure and venous outflow from the jejunum obtained simultaneously to the elimination of  $Kr^{85}$ . For details, see text.

Component I		Component II		Component III		Component IV	Venous outflow ml/min · 100 g
$T^{1/2}$ , min	% initial activity	$T^{1/2}$ , min	% initial activity	$T^{1/2}$ , min	% initial activity	$T^{1/2}$ , min	
0.11	34	2.10	23	7.80	43	56.2	14
0.11	27	2.45	17	6.30	56	180	15
0.05	44	2.05	39	6.60	17	57.0	24
0.07	37	1.80	39	8.90	24	36.2	24
0.08	47	1.51	35	5.00	18	36.1	26
0.18	49	1.40	29	4.35	22	100	36*
0.08	37	1.32	40	5.45	23	41.3	37
0.08	58	1.35	25	3.95	17	46.6	38
0.18	42	1.03	32	3.50	26	45.4	43*
0.12	42	1.14	21	3.20	37	20.8	46*
0.08	22	0.96	53	3.10	25	60.8	48*

\* Denotes experimental runs during constant intravenous infusion of isopropylnoradrenalin.

resolved into 4 monoexponential components. Their localization and functional significance are, as yet, not completely known but experiments are in progress to elucidate these problems. A few comments will, however, be made.

The very slow component (IV) was never recorded by the G-M tubes registering activity from the intestine only. Experiments also suggest that the decay curve of  $\gamma$ -radiation becomes monoexponential when the  $\beta$ -radiation has returned to background level. This indicates that the fourth component is located outside the intestinal wall.

Half-time values similar to those of component III have been recorded when  $Kr^{85}$  is locally injected to the intestinal muscularis. Furthermore, assuming a tissue/blood

partition coefficient close to one<sup>5</sup>, the slope of the third component indicates a regional blood flow of 10–20 ml/min · 100 g, which is in close agreement with blood flow values reported for other smooth muscle tissues<sup>6,7</sup>. This data, then, supports the idea that the third component reflects blood flow in the muscularis of the intestine.

<sup>5</sup> J. LADEFOGED, personal communication.  
<sup>6</sup> O. MUNCK, H. LYSGAARD, G. PONTONNIER, H. LEFÈVRE, and N. A. LASSEN, *Lancet* 1, 1421 (1964).  
<sup>7</sup> M. KAMPP and O. LUNDGREN, to be published.

Assuming homogenous flow in the muscularis, the two fast components (I, II) must, by exclusion, be located in the mucosal and submucosal layers. If the tissue/blood partition coefficient is close to one the half-time values of components I and II correspond to blood flows of 400–1400 and 30–70 ml/min · 100 g, respectively.

The fastest component (I) may indicate the existence of a shunting mechanism which would then be of considerable magnitude judging by the percentage of initial activity (Table). Theoretically, the shunting of krypton could be explained in terms of an arterio-venous blood flow shunt, but it is a priori unlikely that as much as 35–50% of the intestinal blood flow should be distributed to such a bypass and there are no other data to support this possibility. Another explanation seems more plausible. Preliminary experiments in this laboratory indicate the existence of a counter-current exchange between the arterial and venous ends of the hairpin-like vascular loops of the intestinal villi<sup>8</sup>. Such an arrangement would facilitate a diffusion transfer from the arterial to the venous end of easily diffusible and/or lipid-soluble substances in the blood. These substances would, instead of passing along the long axis of the vessels, tend to be shunted between the arterial and venous ends of capillary loops, as is the case in the renal medulla<sup>9,10</sup>. It is suggested that

intraarterially injected krypton is shunted in the intestine of the cat by such a mechanism<sup>11</sup>.

**Zusammenfassung.** Der zeitliche Verlauf der Elimination von intraarteriell injiziertem Krypton aus den Geweben des Katzendarmes wurde untersucht. Die gefundene Eliminationskurve konnte in vier Teilkurven aufgelöst und die anatomische Lokalisierung und funktionelle Bedeutung dieser Komponenten kurz diskutiert werden.

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<sup>8</sup> B. FOLKOW, B. LISANDER, and O. LUNDGREN, unpublished observations on the blood flow of the muscularis of the cat bladder.

<sup>9</sup> K. AUKLAND, J. Oslo Cy Hosp 14, 115 (1964).

<sup>10</sup> A. F. LEVER, Acta med. scand. 178, suppl. 434 (1965).

<sup>11</sup> This study was supported by grants from the Medical Faculty, University of Göteborg, from the Air Force School of Aerospace Medicine under Contract AF 61(052)-732 through the European Office of Aerospace Research (OAR), United States Air Force and from the U.S. Public Health Service (HE-05675-04-05).

## STUDIORUM PROGRESSUS

### The Effect of Acute Starvation on Thyroid Function in Rodents

Atrophy and colloid retention in the thyroid as a consequence of starvation was first reported by JACKSON in 1916<sup>1</sup>; STEPHENS<sup>2</sup> reported similar results in 1940. In 1949, WILLIAMS et al.<sup>3</sup> demonstrated diminished <sup>131</sup>I uptake by the thyroid gland in the starved animal. RIVERO-FONTAN et al.<sup>4</sup> reported comparable results. However, VAN MIDDLESWORTH<sup>5,6</sup> and MONEY<sup>7</sup> found diminished uptake by the thyroid in starvation. DONATI et al.<sup>8</sup> in 1963 reported diminished oxygen utilization, decreased erythrocyte radiotriiodothyronine uptake, diminished plasma levels of triiodothyronine and thyroxine, as well as increases in the 24 h <sup>131</sup>iodide thyroid uptake and thyroid concentrations of iodide, moniodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T<sub>3</sub>) and thyroxine (T<sub>4</sub>) in acutely starved rats. The explanation of these results was not immediately apparent, and the present study was undertaken to clarify the mechanisms involved.

**Materials and methods.** Female Sprague-Dawley rats weighing 180–200 g were utilized in all experiments. Starvation was for a 96 h period with water ad libitum. TSH treated rats received 0.5 unit bovine TSH/0.5 ml 0.9% saline solution intraperitoneally at the same time intervals. Litter mates fed a diet of Purina Laboratory Chow served as controls. Oxygen uptake was determined by means of 1 min oxygen uptake spirometer using 100% oxygen with anhydrous potassium hydroxide as the CO<sub>2</sub> absorber. The erythrocyte radiotriiodothyronine uptake

(ET<sub>3</sub>) was determined by a modification<sup>8</sup> of the method of HAMOLSKY et al.<sup>9</sup>

24 h following the administration of 1 µc of carrier free Na <sup>131</sup>I the amount of radioactivity in surgically removed rat thyroids was measured and the thyroid uptake calculated. 4 thyroid glands were subsequently homogenized and hydrolyzed for a 40 h period at 37°C in a buffered solution with pancreatin added to a final concentration of 1% at a pH of 8.5. Aliquots of the hydrolysate and a non-radioactive concentrate of 50 µg each of MIT, DIT, T<sub>3</sub>, T<sub>4</sub>, and potassium iodide were then applied to Whatman No. 1 chromatography paper. Bidimensional ascending chromatography was carried out for 18 h at 20°C in an *n*-butanol dioxane, 2 normal NH<sub>4</sub>OH system

<sup>1</sup> C. M. JACKSON, Am. J. Anat. 19, 305 (1916).

<sup>2</sup> D. J. STEPHENS, Endocrinology 26, 485 (1940).

<sup>3</sup> R. H. WILLIAMS, H. JAFFE, and C. KEMP, Am. J. Physiol. 159, 291 (1949).

<sup>4</sup> J. RIVERO-FONTAN, K. E. PASCHKIS, E. WEST, and A. CATAROW, Endocrinology 51, 100 (1952).

<sup>5</sup> L. VAN MIDDLESWORTH, Fedn Proc. Am. Soc. exp. Biol. 10, 140 (1951).

<sup>6</sup> L. VAN MIDDLESWORTH and M. M. BERRY, Am. J. Physiol. 167, 576 (1951).

<sup>7</sup> W. L. MONEY, Brookhaven Symp. Biol. 7, 137 (1954).

<sup>8</sup> R. M. DONATI, M. A. WARNECKE, and N. I. GALLAGHER, Metabolism 12, 833 (1963).

<sup>9</sup> M. W. HAMOLSKY, M. STEIN, and A. S. FREEDBERG, J. clin. Endocr. 17, 33 (1957).