LOCAL RECIRCULATION OF $^{133}\text{XENON}$ AND $^{85}\text{KRYPTON}$ TO THE TESTES AND THE CAPUT EPIDIDYIMIDIS IN RATS

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Summary. In the rat, the arteries supplying the testis and the caput epididymidis originate in the internal spermatic artery, and the blood vessels to the cauda epididymidis originate in the vasal artery.

This anatomical configuration may explain results obtained in experiments *in vitro* showing that gas originating from injections of saline solutions of $^{133}\text{xenon}$ and $^{85}\text{krypton}$ into the testis was found within 60 sec in the caput epididymidis but not in the cauda or in the same contralateral organs.

In experiments *in vivo* using $^{85}\text{krypton}$ and a microminiature Geiger-Muller tube placed inside the organs, the same type of recirculation was indicated, as well as recirculation from the caput epididymidis to the testis. Ligation of the ductuli efferentes does not change the observation.

Radioactivity increased during the first five min after the injection. The washout of the gas was slow: 94% to 75% of the initial activity remained after 25 min.

Both types of experiments support the hypothesis that exchange of gas, like that of heat, can take place in a countercurrent system formed by the pampiniform plexus and the internal spermatic artery.

INTRODUCTION

In order that the testis may benefit from scrotal thermoregulation, the arterial blood coming to the testes is cooled by the venous blood in the spermatic cord (Waites, 1970) where the veins in the pampiniform plexus intimately surround the internal spermatic artery (Greene, 1968). It is also a possibility that substances like CO_2 and testosterone produced in the testes can pass from the veins to the artery, although such a transport has never been proved (Setchell, 1970).

The arterial blood supply both to the testis and to the caput epididymidis of the rat comes from the internal spermatic artery, while the arteries to the cauda epididymidis originate from the vasal artery. The blood supply to the corpus may come from both sources (Kormano, 1967; Greene, 1968). A counter-current exchange in the pampiniform plexus would, therefore, be highly probable if a locally applied substance could recirculate from the testis to

the caput epididymidis without appearing in the ipsilateral cauda and corpus epididymidis or the contralateral testis and epididymidis.

The present experiment was carried out to explore the possibility of recirculation of gases from the testes to the caput by way of the blood vessels. This being a passive process, it could only depend on concentration gradients. Inert gases ^{133}Xe and ^{85}Kr were selected since only a small percentage is left in the oxygenated blood after passage through the human lung (Chidsey, Fritts, Hardewig, Richards & Cournand, 1959). The gases would not interfere with metabolic processes, and the beta-emission from ^{85}Kr permits the measurement of changes in the concentration in very distinct and small areas. The 1% gamma emission from ^{85}Kr will only provide a small counting error from more distant gas when beta-radiation is measured, but it is, on the other hand, sufficiently strong to permit external measurement of the radioactivity. The gases used therefore are convenient markers, but they are not normally present in the body, and if the recirculation proposed is to have physiological significance, then it must also be shown to occur with physiologically important compounds.

MATERIALS AND METHODS

Two methods were used to ascertain the recirculation of the inert gases: (1) a dynamic *in-vivo* method, and (2) a static counting of radioactivity in organs to which blood circulation was stopped 60 sec after the injection of the gas.

Measurement in vivo

Mature Charles River rats in use for breeding purposes several times a week were anaesthetized by injection of 30 mg/kg Mebumal (Nembutal). The body temperature of the animals was kept at about 38°C by electric light bulbs before and during the experiment. At laparotomy, the right testis and epididymis were delivered out of the abdomen, and the ductuli efferentes were ligated with 4-0 catgut, taking care to avoid the major blood vessels. A 0.03-ml vol. of a saline solution of ^{85}Kr (5 mCi/ml) was injected through a 26-G needle into the testes of five animals, and the radioactivity on the surface of the caput epididymidis was measured during the following 30 min by placing a cylindrical microminiature, halogen-quenched, stainless steel Geiger-Muller tube (diameter 1.5 mm, length of window 8 mm, wall thickness 29 mg/cm², EON model 5107) between the head and the epididymal fat. The probe was also inserted into the caput epididymidis, and into the testis on occasions when the gas was injected into the caput. The probe was inserted into the tissue through a small incision in the capsule. The signals from the probe were counted and printed by a modified detector for ocular tumours (DOT) (EON Corporation, Brooklyn). In the first 5 min after the injection, the counting period was 10 sec, but later it was changed to 1 min.

Measurement in vitro

In ten similarly anaesthetized animals operated on as described above, the spermatic cords, including the internal spermatic artery, were clamped on both

sides 60 sec after injection of 0.1 ml ^{133}Xe (2 mCi/ml) or ^{85}Kr (5 mCi/ml) solution into the testes. Immediately after clamping the caput, the corpus and the cauda epididymidis from both sides were dissected and placed on separate glass slides and covered by plastic tape (Scotch No. 810, Minnesota Mining & Manufacturing Co.). The testes from both sides were quick-frozen by spraying with Cryokwik TM and temporarily placed on dry carbon dioxide. Each testis was cut into eight to ten sections by a cryostat; the sections were placed on glass slides and covered with tape.

The radioactivity in each slide was counted for 60 sec by a shielded miniature Geiger-Muller tube (aperture diameter 5 mm, height of collimator 5 mm) connected to the DOT counter and printer. All slides were counted within 1 hr after the injection of the gas solution.

RESULTS

All injections, *in vivo*, of krypton-saline into the testis resulted in an increase in radioactivity in the ipsilateral caput epididymidis during the first 5 min from background levels of 0 to 2 ct/min up to 84 ± 40 ct/min (mean $\pm$ S.E.M.) When the probe was placed inside the caput and both the efferent ducts and all or most of the lymphatic vessels ligated (by ligation of the whole septum between the testes and the caput epididymidis), the activity was 35 ± 5 ct/min. The activity measured in the testis when krypton was injected into the caput was 130 ± 5 ct/min. The activity measured in the testis when krypton was injected into the corpus was 130 ± 91 ct/min. These values are all significantly different from those obtained when krypton was injected into the cauda epididymidis ($P < 0.005$, Student's *t* test). If krypton was injected into the cauda epididymidis, a ligature was placed around the corpus, and the probe was placed in the testicular tissue, the counts registered were 4 ± 1 ct/min.

The pattern of radioactivity measured after injection of the gas-saline into the caput epididymidis was very similar to that from the testis during the 30-min period after injection (Text-fig. 1). The activity increased during the first 5 min after the injection, but later a slow decrease started. At 30 min after the injection, however, 94% to 75% of the radioactivity recorded at 5 min was still present.

The radioactivity measured *in vitro* from the caput epididymidis was higher than the radioactivity measured from any of the contralateral organs in the five animals injected with ^{133}Xe and the five injected with ^{85}Kr (Table 1). The radioactivity measured from the corpora was higher than the background level, while the radioactivity from the ten caudae was at the background level. The difference between counts obtained from corpora and caudae was small, but significant ($P < 0.01$). The counts from the caput had as a mean 7.4% (range 1.0 to 18.3) of the maximal count per minute obtained in any of the testis slices from the same animal, and 3.1% (0.5 to 7.4) of the radioactivity found in all the testis slides.

The results using krypton and xenon were similar.

The counts obtained from the testis slices varied very much within the same testis; in most instances, there was a low level followed by an increase until the

TEXT-FIG. 1. Radioactivity measured in the testis or the caput epididymidis of rats after countercurrent exchange of ^{85}Kr between the cord vessels. Each curve represents the mean of counts from five animals, from 6 min on as mean of 5-min periods. The counts were calculated for each animal as relative values of the maximal count obtained at any time during the experiment. $\circ$, The probe placed in the head of the epididymis: krypton-saline injected into the testis. $\times$, The probe placed on the surface of the head: krypton-saline injected into the testis. $\square$, The probe placed in the testes: krypton-saline injected into the caput epididymidis. —, Theoretical clearance curve after local injection if the blood flow was 16 ml/100 g/min.

maximal counts from the slices were obtained from the region of injection. The minimum count stated for testes was almost always obtained in the very first or last (and sometimes very thin and small) slice of the testis.

As xenon and krypton are gases and can escape from the organs during the handling, even when blood circulation is stopped, the counts recorded only

Table 1. Radioactivity of the epididymis and the testis after injection of 0.1 ml ^{133}Xe or ^{85}Kr -saline into rat testes.

Inert gas	Epididymis			Testis slice		Testis total counts	$\frac{\text{Caput} \times 100\%}{\text{Max. testes}}$	$\frac{\text{Caput} \times 100\%}{\text{Whole testis}}$
				Maximum count	Minimum count			
	Caput	Corpus	Cauda					
¹³³ Xenon	6	3	2	371	5	549	1.6	1.1
	34	6	0	186	6	459	18.3	7.4
	8	2	1	90	17	432	8.9	1.9
	17	2	1	177	0	252	9.6	6.7
	22	1	0	292	0	594	7.5	3.7
⁸⁵ Krypton	17	3	0	1775	4	3454	1.0	0.5
	31	7	2	1808	3	2886	1.7	1.1
	164	4	2	1870	4	3196	8.8	5.1
	265	14	4	3130	2	9120	8.5	2.9
	87	15	3	4672	7	11574	1.9	0.8

Values expressed as counts per minute. The spermatic cord was clamped 60 sec after the injection in all ten animals.

represent the relative situation. Recounting of the head after counting all the organs showed that 25 to 75% of the activity was left.

DISCUSSION

Our experiments suggest that, in the rat, there is a countercurrent mechanism for exchange of gases in the vascular supply to the testes and the caput epididymidis. The corpus seems also to be involved but to a lesser degree. The recirculation of the gases could be shown even when the ductuli efferentes and the testicular lymph vessels were ligated. In the animals with ligations, the only intact way left is the blood vascular system. A recirculation based on gas in aortic blood can be excluded because of the low levels in the contralateral organs.

The heat exchange between the testicular venous and arterial blood takes place in the pampiniform plexus (Waites, 1970). The present experiments and the anatomy of the blood vessels strongly indicate that the gas exchange also takes place in this region in the rat. Under normal conditions, Setchell & Hinks (1969) were unable to show exchange of O_2 , CO_2 , hydrogen ions or glucose in rams and marsupials. During anaesthesia with high P_{O_2} and P_{CO_2} values, indications were found, however, that exchange of O_2 and CO_2 could take place.

The artery to the caput epididymidis originates from the internal spermatic artery, while the arteries to the cauda originate from the vasal artery.

This may explain why activity can be found in the caput but not in the cauda. Anastomoses between branches from the internal spermatic artery and the vasal artery (Kormano, 1967; Greene, 1968) explain the small but significant increase in the radioactivity in the corpus epididymidis. The increase may, theoretically, represent diffusion of the gases within the lumen of the epididymal tube; however, this seems less probable considering the length of this coiling tube. The radioactivity measured when placing the probe between the epididymal fat pad and the caput may partly originate from $^{85}\text{krypton}$ in the fat pad, as the artery to this organ also originates from the internal spermatic artery.

Earlier values for testicular blood flow in the rat calculated on the basis of the clearance of local or intra-arterial $^{133}\text{xenon}$ or $^{85}\text{krypton}$ administration may need correction because one of the principal assumptions for the calculation is that the arterial blood concentration is zero during the measuring period, which may not be valid in this species because of the countercurrent exchange.

Published results on rats (Table 1, Setchell, 1970) suggest a testicular blood flow of 12.0 ml/100 g/min measured with $^{85}\text{krypton}$ and 18.2, 21.0, 20.8 and 17.0 with an indicator fractionating method (the investigations performed partly by the same investigators). This too indicates that testicular flow measurement with the gas clearance method may give values somewhat too low.

The blood flow from the $t/2$ for the increase of radioactivity during the first 60 to 300 sec after the injection was calculated by the formula normally used for flow calculations based on $^{85}\text{krypton}$ clearance

$$\text{Flow} = \frac{\ln 2 \times 0.85}{t/2} \text{ ml/100 g/min.}$$

The mean testicular blood flow was 20 ml/100 g/min and the caput blood flow was 18 and 16 ml/100 g/min with the probe placed in the caput and on the surface, respectively. Even if the correctness of the calculation is unproven, the values found for testis are astonishingly similar to the results mentioned above.

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