

## Determination of Intrapenial Blood Volume Using $^{99m}\text{Tc}$ -Labeled Autologous Red Blood Cells

MASAFUMI SHIRAI, MAMORU NAKAMURA,\* NOBUHISA ISHII,  
SHIRO MITSUKAWA and YOSHIKAZU SAWAI\*

*Department of Urology and \*Department of Radiology,  
Tohoku University School of Medicine, Sendai*

SHIRAI, M., NAKAMURA, M., ISHII, N., MITSUKAWA, S. and SAWAI, Y. *Determination of Intrapenial Blood Volume Using  $^{99m}\text{Tc}$ -Labeled Autologous Red Blood Cells.* Tohoku J. exp. Med., 1976, 120 (4), 377-383 — In 17 impotent patients, radioisotope penography was performed using  $^{99m}\text{Tc}$ -red blood cells (the patient's own red blood cells labeled with  $^{99m}\text{Tc}$ ) for the quantitative analysis of intrapenial blood volume. A visual sexual stimulation (VSS) was given to the patient after injecting the  $^{99m}\text{Tc}$ -red blood cells. Patients showing a complete erection had their intrapenial blood volumes 4.2-11.2 times greater than before VSS (mean increase, 8.0 times). In cases of incomplete erection after VSS the intrapenial blood volumes were 3.3-7.0 times greater than before VSS (mean increase, 4.9 times). In cases showing a gentle rise in their penogram curves without evidence of an erection, intrapenial blood volumes after VSS were 2.0-3.3 times those before VSS (mean increase, 2.9 times). By contrast, in cases showing no response to the VSS or no rise in penogram curve, post-VSS increases in intrapenial pool of blood were very slight, only 1.4-1.7 times the original volume of blood. — blood volume; impotence; penis;  $^{99m}\text{Tc}$ -red blood cells

As a diagnostic technique for discriminating organic from functional impotence, we have developed "radioisotope penography" to identify the features of intrapenial blood flow in patients (Shirai and Nakamura 1970, 1971, 1973, 1975; Shirai et al. 1973). As an isotope for our penography, we previously used simply  $^{131}\text{I}$ -human serum albumin,  $^{113m}\text{In}$ -microcolloid, or  $^{99m}\text{TcO}_4^-$ . But recently a unique method (Eckelman et al. 1971) using  $^{99m}\text{Tc}$ -labeled autologous red blood cells has been developed, which is apparently preferable to any of the foregoing isotopes in quantitating intrapenial blood.

This is a report on our radioisotope penographies using  $^{99m}\text{Tc}$ -labeled autologous red blood cells and some of the clinical results obtained.

### MATERIALS AND METHODS

The subjects were 17 patients whose chief complaint was impotence, ranging in age between 18 and 56 years, all seen in our outpatient clinic (Table 1). Only 2 of the 17 patients (Patients 4 and 12) were not organically impotent. In the other 15 patients, 9 (Patients 2, 6, 8, 9, 10, 11, 13, 15 and 16) had a history of trauma or had previously had at least one operation, 1 (Patient 1) had diabetes mellitus, 1 (Patient 3) intervertebral disc

---

Received for publication, July 26, 1976.

TABLE 1. *Impotence cases*

Case No.	Name	Age	Condition	Isotope dose ( $\mu\text{Ci}$ )	Rate of increase ( $\times$ original volume)	Blood volume pooled in the penis in response to VSS (ml)	Penogram pattern
1	K.S.	27	Diabetes mellitus	277	4.2	16.9	B*
2	K.I.	45	Trauma of head and neck	242	7.0	28.1	B
3	H.S.	30	Hernia of intervertebral disc (before op.)	302	2.0	8.0	A
4	Y.O.	26		365	11.2	44.7	B*
5	A.K.	26	Orchitis, nephritis	311	1.4	5.5	No response
6	F.T.	28	Fracture of the penis	495	9.9	39.5	B*
7	T.T.	35	Irradiation of external genitalia	241	3.7	14.8	B
8	H.Sy.	56	TURpt	180	3.1	12.5	A
9	H.A.	18	Fracture of pelvis, urethral rupture	295	3.3	13.1	A
10	Y.S.	51	Hernia of intervertebral disc (after op.)	254	3.9	15.6	B
11	T.O.	39	Comp. fracture of L <sub>4</sub> , L <sub>5</sub>	298	1.7	6.9	No response
12	S.M.	28	Honey-moon condition	362	8.2	32.8	B*
13	T.K.	46	Trauma of neck (C <sub>5</sub> , C <sub>6</sub> )	371	9.1	36.3	B*
14	T.Ko.	28	Chronic prostatitis	299	3.1	12.3	A
15	K.U.	40	Trauma of neck	271	6.7	26.8	B
16	T.N.	41	Contusion of perineal region	251	3.3	13.1	B
17	C.M.	35	Klinefelter's syndrome (XXY/47)	285	5.1	20.3	B*

\* Cases of complete erection. VSS: visual sexual stimulation.

A type: a rising curve but no erection in response to VSS

B type: a prominent rise in the penogram curve with an accompanying complete or incomplete erection.

No response type: no rise in the penogram curve.

hernia, and 1 (Patient 14) chronic prostatitis; the remaining 3 cases were: Patient 5 under close examination for orchitis, proteinuria and dysuria, Patient 7 with irradiation of the external genitalia, and Patient 17 with Klinefelter's syndrome with chromosomal arrangement of XXY.

The  $^{99\text{m}}\text{Tc}$ -red blood cell kit prepared by CEA IRE SORIN (CIS) was used as  $^{99\text{m}}\text{Tc}$  to label the autologous red blood cells in our radioisotope penography.

Two ml of the patient's blood was sampled using a heparin-containing syringe and put into a sterilized tube. This was mixed with 0.5 ml of a reduction agent consisting of stannous pyrophosphate, sodium pyrophosphate, and sodium chloride. It was incubated at room temperature for 5 min (stirred 3-4 times). Then, after 10-min centrifugation at 2000 rpm, the upper plasmal layer was completely discarded. About 500  $\mu\text{Ci}$  of  $^{99\text{m}}\text{TcO}_4^-$  was added and the preparation was incubated again at room temperature for 5 min, while it was gently stirred occasionally. This was again centrifuged for 10 min at 2000 rpm and the upper layer was discarded. Then, 1 ml of physiological saline or the patient's plasma was added, and the mixture was thoroughly stirred. An appropriate dose of this final

preparation was intravenously injected into the patient. The "appropriate dose" varied individually, ranging 180–495  $\mu\text{Ci}$ , with a mean of 229.9  $\mu\text{Ci}$ . To measure the systemic stabilization of  $^{99\text{m}}\text{Tc}$ -red blood cells after loading, we sampled the blood of the patient within 5 min of injection and subjected it to paper chromatography using an 85% methanol solution. The  $^{99\text{m}}\text{Tc}$ -red blood cells were found to be fairly stable with their rate of liberation in the blood ranging only 3.2–5.5%. Also favorable was their high labeling rate of 93–94%.

The apparatus employed was a Hitachi 4-channel kinetographic recorder, which had been usually used in renography, connected to a data-dealing minicomputer (Hitac-10 8 K words). The collimator used, however, was one we had developed.

The patient was placed in a supine position with the penis inserted as deep as possible into the collimator to avoid exogenous  $\gamma$  rays. A control collimator was placed directly above the pubic region to determine the penial background blood flow.

When the penogram curve after loading the  $^{99\text{m}}\text{Tc}$ -red blood cells became flat, a visual sexual stimulation (VSS) was given to the patient and changes in the curve were studied.

The penogram curves changed in response to the VSS and were sorted into 3 patterns: A type, showing a gentle rise in the curve without sensation of erection; B type, showing a precipitous rise in the curve accompanied by erection; and the no-response or organic type, without any tendency toward rise in the curve (Shirai and Nakamura 1975).

After confirming that  $^{99\text{m}}\text{Tc}$ -red blood cells had permeated the body, 4 ml of the patient's blood was taken with a syringe. The blood in the syringe was inserted in a third collimator at a depth approximately the same as that of the penis inserted into its collimator. The radioactivity of the 4 ml of blood was counted simultaneously with the penogram using the same type collimator. It is essential to use always the same type syringe and always to insert it into its collimator precisely to the same depth.

The radioactivity count from the penis itself, which would represent the radioisotope penogram in the true sense of the term, was determined by subtracting the count of the penial background collimator from that shown on the penogram curve. By dividing this count by the computer-recorded count from the 4 ml of systemic blood and multiplying this quotient by four, we can estimate the volume of blood in the penis. In this way, post-VSS increases in the intrapenial pool of blood, their rate and volume were measured in the 17 impotence patients (Table 1).

## RESULTS

The post-VSS penogram pattern for 11 of the 17 cases (Table 1) was of B type, including 6 cases of fullfledged erections. The curve pattern of these 6 cases was distinctly characteristic in that the curve did not follow a constant rising course but became flat in due time (Fig. 1). The flattening of the curve apparently indicates an equilibrated state of blood flow, the penis fully filled with blood coinciding with complete erection. Four cases had A-type penogram, including one nearer to the no-response type. The remaining 2 cases showed virtually no-response.

Pairing the penogram patterns with the causes of impotence, we found that the 2 cases (Patients 4 and 12), with no organic causes behind their impotence, had a B-type penogram pattern. The other 15 cases, which had some organic indications of impotence, included 9 B-type, 4 A-type and 2 (Patients 5 and 11) no-response type patients.

The calculation of blood volumes intrapenially pooled after VSS (Table 1) revealed that in the 11 B-type cases the blood volumes in their penises increased

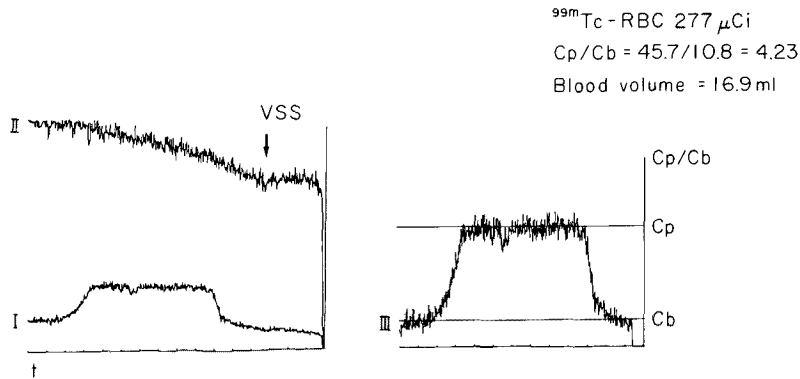


Fig. 1. Penogram curve with  $^{99m}\text{Tc}$ -RBC (Patient 1).

Left: original data. Right: computer processed data. I: The curve is obtained by inserting the penis into the collimator. II: The curve is obtained by putting the collimator over the pubic region. III: The curve is obtained by subtracting background count from penial curve.

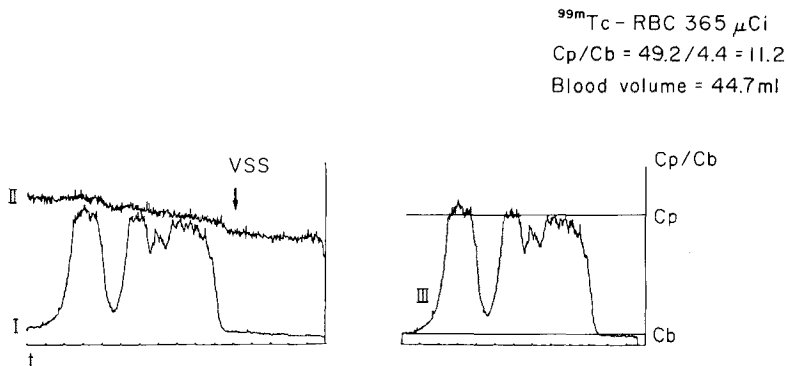


Fig. 2. Penogram curve with complete erection (Patient 4).

Left: original data. Right: computer processed data. I: Penis collimator curve. II: Background collimator curve. III: Penis minus background.

3.3–11.2 times, with a mean of 6.6 times. Among them, 6 cases (Patients 1, 4, 6, 12, 13 and 17) showed complete erection and had 4.2–11.2 times increases in blood volume with a mean of 8.0. The other 5 cases (Patients 2, 7, 10, 15 and 16) of incomplete erection showed 3.3–7.0 times increases, with a mean of 4.9 (Figs. 2 and 3).

Intrapenial blood volumes at the time of complete erection, averaging 32 ml, varied individually between 16.9 ml and 44.7 ml, suggesting that the difference in the size of the penises caused a fluctuation in the results.

In the 4 A-type cases (Patients 3, 8, 9 and 14), intrapenial blood volumes after the VSS were 2.0–3.3 times those before the VSS with a mean increase of 2.9. The post-VSS blood volumes in the penis in these cases were 8–13 ml, averaging 11.5 ml (Fig. 4).

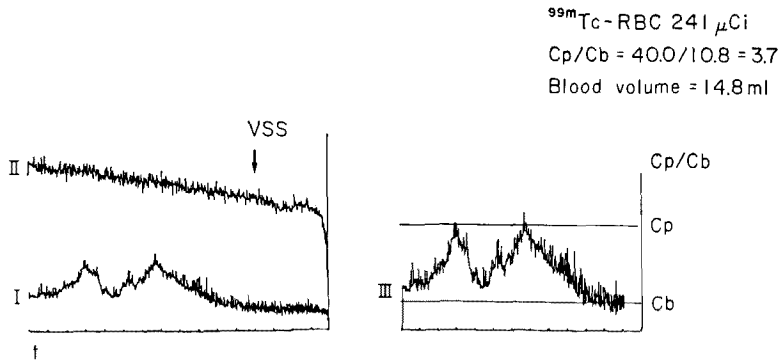


Fig. 3. Penogram curve with incomplete erection (Patient 7).  
 Left: original data. Right: computer processed data. I: Penis collimator curve. II: Background collimator curve. III: Penis minus background.

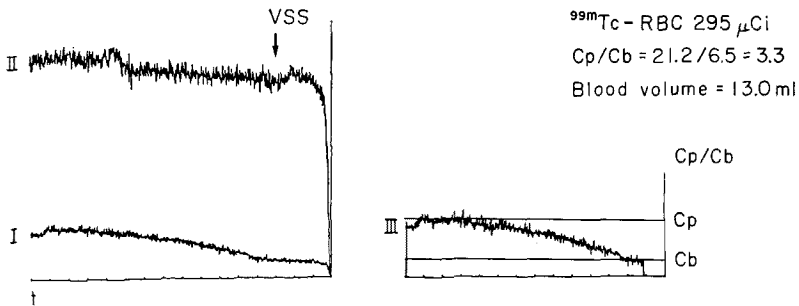


Fig. 4. Penogram curve showing a gentle rise without sensing an erection (Patient 9).  
 Left: original data. Right: computer processed data. I: Penis collimator curve. II: Background collimator curve. III: Penis minus background.

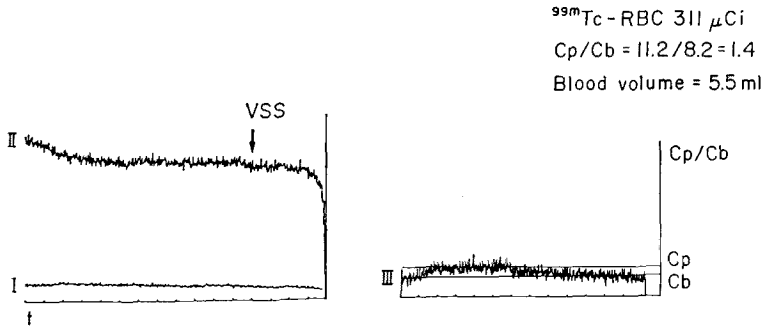


Fig. 5. Penogram curve showing no response to VSS (Patient 5).  
 Left: original data. Right: computer processed data. I: Penis collimator curve. II: Background collimator curve. III: Penis minus background.

The 2 no-response-type cases (Patients 5 and 11) had an average of 6.2 ml post-VSS intrapenial blood volume, showing insignificant increases of 1.4 times and 1.7 times their original volume (Fig. 5).

## DISCUSSION

$^{131}\text{I}$ -human serum albumin,  $^{113\text{m}}\text{In}$ -microcolloid, or  $^{99\text{m}}\text{TcO}_4^-$  has been conventionally used as the isotope for scanning various organs through blood-flow features. Recent development of a unique method using  $^{99\text{m}}\text{Tc}$ -labeled autologous red blood cells has apparently paved the way for more favorable determination of the blood volume in any specific portion of the body.

The effective half-life of 4.5 hr of  $^{99\text{m}}\text{Tc}$ -red blood cells makes it suitable for making various types of measurements, while at 2.7 mrad/mCi (Ryo et al. 1974) for the whole-body, the low radiation of this isotope allows individual mass dosing.

In the present study we used the CIS-produced  $^{99\text{m}}\text{Tc}$ -red blood cell kit. This agent is easy to manage and has a high rate of  $^{99\text{m}}\text{Tc}$  labeling on red blood cells ranging 93–94%, which is much more favorable than 57–70% noted by Korubin et al. (1972), and 60% labeling noted by Atkins et al. (1973).  $^{99\text{m}}\text{Tc}$ -liberation rate from the red blood cells was reported by Ryo et al. (1974) to be 2% in 90 min and 50% in 24 hr. In our test, the  $^{99\text{m}}\text{Tc}$  liberation rate 5 min after loading was 2.4–5.5%, showing high stability of  $^{99\text{m}}\text{Tc}$ .

In the present radioisotope penography performed in 17 impotent patients, the doses of  $^{99\text{m}}\text{Tc}$ -red blood cells individually varied from 180  $\mu\text{Ci}$  to 495  $\mu\text{Ci}$ . So, we measured intrapenial blood volumes on the basis of radioactivity counts from a fixed volume of blood. As a result, it was found that the volumes of blood in the penis at the time of complete erection were 4.2–11.2 times larger than before erection; the volumes of blood at that time ranged 19.6–44.7 ml. It is very interesting that complete erection of the penis causes the penogram curve to reach a plateau, a phenomenon specific to the use of  $^{99\text{m}}\text{Tc}$ -red blood cells. As yet the reason for this unique reaction is unknown. Since the patient's own red blood cells are labeled with the isotope, it is probable that the penogram curve indicates exactly the hemodynamic state in the penis. The flattening of the curve at the time of full erection seems to show that the inflow and outflow of blood has reached an equilibrium in the penis. This penogram curve pattern resulted in each case where complete erection occurred. But the volume of blood in the penis varies individually as stated before, apparently due to differences in penis size. In this regard, the use of  $^{99\text{m}}\text{Tc}$ -red blood cells seems the most appropriate for showing in detail the intrapenial blood volume exchange during a full erection. It may even be possible to estimate reversely the size of the penis by determining the intrapenial blood volumes at the time of full erection.

The intrapenial pool of blood after VSS when compared with the penogram patterns shows that the volume of blood is largest in the B-type group and least in the no-response-type group. Since intrapenial blood volumes individually vary considerably due to differences in the size of the penises at erection, the effects of VSS on penial blood flow can be evaluated more properly by checking the rate of increase than by observing the change in the volume itself. In the B-type penogram group, the cases which exhibited complete erection had intrapenial blood volumes 4.2–11.2 times larger after VSS than before, with a mean increase

of 8.0 times. In cases of incomplete erection, blood volume increased 3.3–7.0 times, with a mean of 4.9 times. In the cases of the A-type group, the increase in blood volume was 2.0–3.3 times, with the mean of 2.9 times. Even in the 2 no-response cases, post-VSS increases in intrapenial blood volume could be observed, though the rates were very slight, being 1.4 times and 1.7 times. We are now planning to conduct studies on a larger group of patients to obtain satisfactory data that may help us to establish diagnostic criteria for distinguishing organic from functional impotence.

The data obtained in the present radioisotope penography for quantitative analysis of blood volume may support our recommendation that the  $^{99m}\text{Tc}$ -red blood cells technique, simple in procedure, should be used extensively in determining circulating blood-flow conditions and in imaging blood-pooling organs.

#### References

- 1) Atkins, H.L., Eckelman, W.C., Klopper, J.F. & Richards, P. (1973) Vascular imaging with  $^{99m}\text{Tc}$ -red blood cells. *Radiol.*, **106**, 357–360.
  - 2) Eckelman, W.C., Richards, P., Hauser, W. & Atkins, H.L. (1971) Technetium-labeled red blood cells. *J. nucl. Med.*, **12**, 22–24.
  - 3) Korubin, V., Maisey, M.N. & McIntyre, P.A. (1972) Evaluation of technetium-labeled red blood cells for determination of red cell volume in man. *J. nucl. Med.*, **13**, 760–762.
  - 4) Ryo, U.Y., Lee, J.I., Zarnow, H., Schwartz, M.P. & Pinsky, S. (1974) Radionuclide angiography with  $^{99m}\text{Tc}$ -labeled red blood cells. *J. nucl. Med.*, **15**, 1014–1017.
  - 5) Shirai, M. & Nakamura, M. (1970) Differential diagnosis of organic and functional impotence by the use of  $^{131}\text{I}$ -human serum albumin. *Tohoku J. exp. Med.*, **101**, 317–324.
  - 6) Shirai, M. & Nakamura, M. (1971) Radioisotope penogram by means of  $^{113m}\text{In}$ -microcolloid. *Tohoku J. exp. Med.*, **105**, 137–140.
  - 7) Shirai, M. & Nakamura, M. (1973) A study on the radioisotope penogram in the dog. *Tohoku J. exp. Med.*, **111**, 179–186.
  - 8) Shirai, M. & Nakamura, M. (1975) Diagnostic discrimination between organic and functional impotence by radioisotope penogram with  $^{99m}\text{TcO}_4^-$ . *Tohoku J. exp. Med.*, **116**, 9–15.
  - 9) Shirai, M., Nakamura, M. & Matsuda, S. (1973) Differential diagnosis between functional and organic impotence by radioisotope penogram following visual sexual stimulation. *Tohoku J. exp. Med.*, **111**, 187–195.
-