[Chem. Pharm. Bull. 16(6)1062—1064(1968)]

UDC 615.034 : 547.584.02

Synthesis of Radioactive Sodium 5-Acetamido-2,4,6-triiodo-N-methyl-isophthalamate (^{131}I) and Its Application to External Measurement of Glomerular Filtration Rate in Rabbits

Sadatake Kato, Kunio Kurata, Takao Wakabayashi, and Yoshihiko Sugisawa

Dainabot Radioisotope Laboratories, Ltd.[1]

(Received August 19, 1967)

Radioactive sodium 5-acetamido-2,4,6-triiodo-N-methyl-isophthalamate(^{131}I)was synthesized by isotopic exchange reaction in acidic aqueous solution. When this compound was injected intravenously into rabbit, it was excreted very rapidly and little of it was taken up by organs except kidney. The blood clearance of the compound obtained by external counting after the single injection was exponential after a certain period of equilibrium and its rate was independent of the administered dose. Those results indicate that this substance is filtered at the glomeruli passively and the half clearance time can be considered to be a parameter of the renal glomerular filtration rate.

In clinical diagnosis, renal glomerular filtration rate (GFR) has been measured with such substance as inulin or mannitol which is filtered at the glomeruli but is neither reabsorbed nor excreted by the tubules. GFR is calculated by the formula $C=UV/P$, in which C is the GFR in ml/min, U is the urinary concentration of the substance in mg/dl, P is the plasma concentration of the substance in mg/dl, and V is the urinary flow rate in ml/min.

Recently, E.M. Sigman, *et al.*[2,3] have demonstrated that renal clearance of radioactive sodium 5-acetamido-2,4,6-triiodo-N-methylisophthalamate (^{131}I) (radioactive sodium iothalamate ^{131}I) approximates that of inulin. In this radioactive method, concentrations of the substance in blood and urine samples are quantitated more accurately and easily than in former methods. However, in their constant infusion technique, the plasma concentration of the substance was kept constant by intravenous infusion of the solution of the substance continuously and the plasma and the urine samples had to be withdrawn several times for measuring their radioactivity. Whereas this rather complicated procedure gives a considerable burden to physicians and patients, it still involves some inaccuracy relating the complete sampling of urine from bladder.

Concerning those, we considered as follows. If the injected sodium iothalamate is excreted only at glomeruli but is neither deposited nor degraded at any organ in a body, the blood clearance rate of sodium iothalamate after the single injection should be exponential and be expressed as $dP/dt = -kP$. In this formula, P is the plasma concentration of this substance and k is considered to be an excretion rate of sodium iothalamate at glomeruli and be obtained as $k=(GFR\times P)/(D\times P)=GFR/D$, where D is the distribution space of sodium iothalamate in the body and $(GFR\times P)$ represents the excreted sodium iothalamate per minutes, because the concentration of the sodium iothalamate in the plasma and glomerular filtrate at a given time are considered to be equal. As GFR is expressed in terms of body weight or body surface area in general, we can consider the value of k itself as the GFR expressed in terms of the sodium iothalamate distribution space of each individual. Then, k should be

1) Location: *334 Minoridai, Matsudo City, Chiba.*
2) E.M. Sigman, C.M. Elwood, M.E. Reagan, A.M. Morris, and A. Catanzaro, *Investigative Urology*, **2**, No. 5, 432 (1965).
3) E.M. Sigman, C.M. Elwood, and F. Knox, *J. of Nucl. Med.*, **7**, 60 (1965).

constant and independent of the plasma concentration of sodium iothalamate. By using radioactive sodium iothalamate (^{131}I), k should be calculated as $k=0.693/T_{1/2}$, where $T_{1/2}$ is its half clearance time obtained from its blood clearance curve measured by the external counting following a single injection of the substance.

In this report, the synthesis of radioactive sodium iothalamate (^{131}I) was at first studied and then its clearance curve was measured to verify our assumption mentioned above.

Experimental

Synthesis of Radioacitve Sodium Iothalamate by Exchange Reaction——To a solution of 30 mg of sodium iothalamate in 1 ml of phosphate buffer of various pH, carrier free sodium radioiodide (^{131}I) was added and refluxed. After 1,3 and 6 hours of refluxing, samples were taken and the rate of the exchange reaction for each refluxing period was determined by ascending paperchromatography, using a mixture of *n*-amyl alcohol: pyridine:water (35:35:30) as a developer. In this system, *Rf* values or sodium iothalamate and iodide are 0.55 and 0.70 respectively. The results are summarized in Table I.

Table I. Effect of pH on Exchange Reaction

Refluxing period / pH	1 hr	3 hr	5 hr
3.86	100[a)]	100	100
5	100	100	100
7	60	80	95
9	—	—	15
12	—	—	—

a) % of Radioactive sodium iothalamate ^{131}I formed.

It was known from the result that this exchange reaction takes place readily at acidic solution. The product was purified by reprecipitations with HCl and finally disolved in aqueous solution containing 2 mg of $NaHCO_3$ per ml. With appropriate stabilizing agents, this product can be used for several weeks with negligible amount of free iodide.

Measurement of the Clearance Curve and the Organ Distribution in Rabbits 1 Hour after the Intravenous Injection

Experimental Condition——Male rabbits, weighing 2.1 to 2.3 kg, were kept from food and water for 24 hours before the experiment. 45 min prior to the injection of radioactive sodium iothalamate (^{131}I), 20 ml of water per kg of body weight was instilled through a stomach tube. The animal was then anesthetized with hexobarbital sodium injection and fixed in supine position. A collimated scintillation probe was directed to a fixed ear (or heart) and the radioactive substance was injected into the another ear vein. After one hour recording of the clearance curve on paper, a blood sample was taken for measuring the radioactivity. The animal was sacrificed by bleeding and the organ distribution of the radioactivity was counted.

Results of the Experiment

I. Distribution of Radioactive Sodium Iothalamate (^{131}I) in Rabbits

The mean values of the radioactivity in each organ are summarized in Table II. The mean value of the blood sample one hour after the injection was 0.034% of the total injected

Table II. Organ Distributions (Mean Values) 1 hr after *i.v.* of Radioactive Sodium Iothalamate ^{131}I into Rabbits

Organ	Kidney	Thyroid	Liver	Spleen
% in total organ	1.32	0.17	1.22	0.013
% per gram of organ	0.086	0.53	0.018	0.013

dose per ml. About 70% of the dose was collected from urine in the bladder. The radioactivity in other organs were negligible.

II. Clearance Rates

Observed clearance curves of the radioactive sodium iothalamte (^{131}I) from blood are given in Fig. 1 and are compared with those of radioactive sodium *o*-iodohippurate (^{131}I) and ^{99m}Tc–Fe–ascorbic acid complex obtained by the same experimental method.

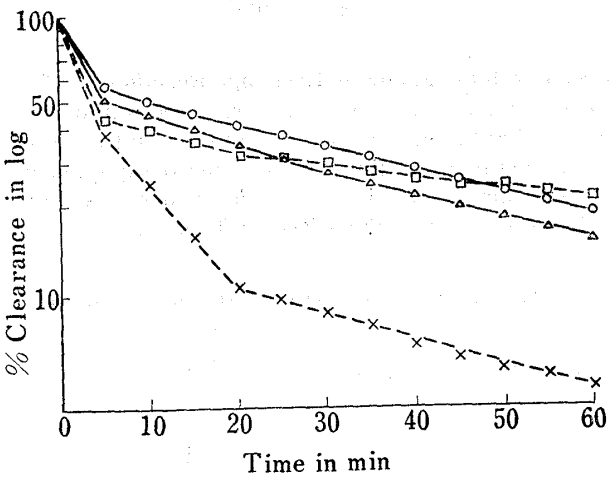

Fig. 1. Comparison of Clearance Rates in Rabbits

	$T_{1/2}$ in min
—○— Sodium iothalamate (0.1 mg)	42.5
—△— Sodium iothalamate (10 mg)	42.5
--□-- ^{99m}Tc—Fe Complex	77.5
--×-- Sodium *o*-iodohippurate	30.0

Discussion

The injected radioactive sodium iothalamate (^{131}I) was excreted very rapidly through kidney into urine and very little was taken up by organs. This extremely rapid excretion promises that this material can be used for clinical diagnosis of kidney function within a short period of time, giving a very little radiation hazard to patients.

It was demonstrated from the clearance curve that this substance is excreted exponential after the equilibrium period, and its clearance rate is independent of the dose administered, at least up to 10 mg in a rabbit.

Those facts indicate that this substance is transported passively at glomeluri, and its clearance is considered to be an index for GFR of the rabbit. Unexponential clearance curve before equilibrium would reflect its expanding distribution space in the body.

In comparison with the excretion rate of radioactive sodium *o*-iodohippurate (^{131}I), which is considered to be a substance excreted at tubules, the half clearance time of the radioactive sodium iothalamate (^{131}I) after the equilibrium period is slower by about 1.4 times than that of radioactive *o*-iodohippurate (^{131}I) in rabbits.