Quantitative analysis of whole body autoradiogram using a computer-assisted image analyzer

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Summary

A computer-assisted image analyzer system for the determination of tissue drug concentration using whole body autoradiograms is described. An image of a whole body autoradiogram was taken by the video camera, and the shading was corrected by the ratio correction procedure. Calibration curve was obtained by using standard tissue sections which contained known concentration of radioactivity. The non-linear relationship between mean gray values obtained by the video analysis method and radioactivity concentration in corresponding tissues was corrected by the linearization process. These correction procedures were effective to solve the problems of video camera-based image analysis of autoradiogram.

By using this video analysis method, indomethacin concentration in rat tissue after p.o. and i.v. administration was determined and was compared with that obtained by scintillation counting method. Both methods gave almost identical results in most of tissues, however, blood level of a drug affected the determination of its concentration in capillary rich tissues in the video analysis method.

Introduction

Recent progress in computer-assisted image analyzers enables us to perform quantitative analyses of brain autoradiogram, such as measurement of local cerebral glucose utilization¹⁻³⁾, local cerebral blood flow⁴⁻⁶⁾, or receptor-ligand binding⁷⁻¹⁰⁾. Although the brain is a complex organ composed of many different structural and functional components, specific gravity of each component is uniform. It is, therefore, relatively easy to obtain concentration of radioactivity in a discrete brain region of interest (ROI) by extrapolating the gray value or optical density of the ROI to the calibration curve. In the case of whole body autoradiography, however, it is more difficult to perform quantitative analyses because specific gravity and radioactivity concentration of each organ vary widely.

The present paper deals with the method of quantitative analysis of whole body autoradiogram using a computer-assisted image analyzer. Various methods for the recording of autoradiogram have been proposed, such as spot densitometry¹¹⁾, rotating-drum densitometry^{7,8)} and video camera system^{9,10)}. In the present study, a video camera system is used for the image recording because this method allows a quick acquisition of images and the magnifying power can be altered easily. Autoradiograms of male rats after p.o. and i.v. administration of radioactive indomethacin were recorded by using the video camera system, and were analyzed by the computer-assisted image analyzer to obtain indomethacin (equivalent) concentration in several tissues. The values obtained by this video analysis method were compared with the values obtained by the conventional scintillation counting method using dissected tissues.

Materials and methods

(1) Materials

Male Sprague-Dawley rats were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). [2-¹⁴C]Indomethacin (14.2 mCi/mmol: radiochemical purity was more than 97%) was obtained from Amersham Japan Co. (Tokyo, Japan). Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Soluene-350 was obtained from Packard Instrument B.V. (Groningen, Netherlands). Sakura tritium type film was obtained from Konishiroku Photo Ind. Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

(2) Drug administration

Standard samples used in the video analysis method were obtained from rats orally administered radioactive indomethacin. Five rats weighing 203 ± 1 g (mean $\pm$ S.E.) were given a single oral dose of [¹⁴C]indomethacin in 0.9% sodium bicarbonate (100 μ Ci/10 mg base/kg). Each rat was then killed 0.5, 1.0, 1.5, 2.0 and 4.0 hr after administration by bleeding from inferior aorta under ether anesthesia. Immediately after sacrificing at each time point, the submaxillary gland, liver, kidney, spleen, pancreas, testis and femoral muscle were removed from each rat. A part of each tissue was separated, weighed and solubilized in soluene-350 at 37 °C overnight followed by the measurement of radioactivity using an Aloka model LSC-903 liquid scintillation spectrometer (Aloka Co., Tokyo, Japan). The other part

of each tissue was cut into thin sections by the same method used in the preparation of whole body sections as described below. They were used for the standard tissue sections for the autoradiographic analysis.

Indomethacin concentration in rat tissues was determined by both video analysis and scintillation counting methods after p.o. and i.v. administration. Oral dosing was performed by the same method as described above. Rats were killed 1 and 2 hr after administration, and were submitted to autoradiographic analysis and scintillation counting of dissected tissues (each 3 animals for each time point). Intravenous injection was performed using [^{14}C] indomethacin dissolved in 0.9% sodium bicarbonate at a dose of 40 $\mu\text{Ci}/1\text{ mg base/kg}$. Rats were killed 15 min and 6 hr after injection, and were used for the examination of indomethacin distribution by video analysis and scintillation counting methods (each 3 animals for each time point).

(3) Autoradiography

Animals or tissues were embedded in 5% carboxymethylcellulose paste, immersed in dry ice-acetone mixture, and 35 μm sections of the frozen samples were taken by using a PMV Type 450 cryo-microtome (PMV Co., Stockholm, Sweden) according to Ullberg's method¹²⁾. The sections were then freeze-dried and exposed to tritium-sensitive films for 4 days at 4°C. The films were developed according to the instructions supplied with the film.

(4) Optical video system

The autoradiographic negatives were placed on a white plastic plate which was back-illuminated by eight glow lamps housed in a metal box. An image of autoradiogram was projected through a 17.5–105 mm, 1:1.8 zoom lens (Fujinon-TV Zoom Lens C6 $\times$ 17.5 B, Fuji Photo Optical Co., Tokyo, Japan) and was taken by a C1000 video camera (Hamamatsu Photonics K.K., Shizuoka, Japan). The video signal, which was digitized 30 times per second in a format of 512 pixels along each of the 480 video lines, was displayed on a 35 cm color display monitor.

(5) Image analyzer

A NEXUS 6400 real time color image processor (Nexus Inc., Tokyo, Japan) was used as an image analyzer. An NEC PC-9801 UV2 computer with 640 kbytes RAM, 4 $\times$ 1 Mbytes floppy disc drives, a color display monitor, a dot matrix printer (NEC Co., Tokyo, Japan) and 2 $\times$ 5 Mbytes cartridge hard disc units (Japan System House Inc., Tokyo, Japan) was connected to the image analyzer, via an IEEE-488 interface. The image analyzer consisted of a 68B09 E central processing unit, an array processor, a video memory with 4 memory planes with 512 $\times$ 512 pixels and 8 bit (256 levels) brightness resolution, a mouse controller and a 35 cm color display monitor.

Operating software written in BASIC and assembler languages was stored in a floppy disc. Images were stored in floppy discs (4 images per disc) or hard disc cartridges (18 images per cartridge).

(6) Image acquisition

After warming up the light source and video camera, 50 video frames of an autoradiogram were accumulated and averaged to produce an 8 bit resolution for each pixel. This step was required to reduce noise. The averaged image of the autoradiogram (original image) was

stored in one of the memory planes. A background (reference) image, i.e. a homogeneous film area was then acquired and averaged by the same method. Shading distortions of the original image was then corrected by the point-to-point ratio correction procedure which was expressed by the following formula :

$$G(x,y)=255-O(x,y)\times 255\times F/R(x,y)$$

where $G(x,y)$ is a gray value of a pixel, $O(x,y)$ is a brightness of an original pixel, $R(x,y)$ is a brightness of a reference pixel and F is a correction factor which shows the ratio of mean brightness between reference and original films. Original and shading-corrected images are shown in Fig. 1.

(7) Calibration curve

Calibration curves were generated by fitting radioactivity concentration (X) and mean gray values (Y) of standard tissues to the following saturation function using a non-linear regression method :

$$Y=S\times(1-e^{-\lambda X^\alpha})$$

where S is a saturation level, λ is a kinetic constant and α is a correction factor which varies by the energy of beta emission from radionuclides, exposure conditions and optical conditions of video system such as iris and sensitivity. When the energy of beta emission is high enough and the thickness of sensitive emulsion is negligible, α falls to one, however, weak beta emitter such as tritium gives decreased α and S values. Elongation of exposure period causes a decrease in α value and an increase in λ value. Regression of latent image (fading) causes an increase in α value. Increased F value (lens closing) of a camera causes a decrease in α value. This correction factor α is quite valuable in determining the relationship between radioactivity concentration and mean gray values of standard sections under various conditions.

(8) Linearization

In order to linearize the non-linear calibration function, the upper limit of gray value S' was introduced. The 90% saturation level of gray value was usually used as the S' value which gave the maximal detectable concentration, $X_{\max}$.

$$Y=S\times(1-e^{-\lambda X^\alpha}) \quad (1)$$

$$S'=S\times(1-e^{-\lambda X_{\max}^\alpha}) \quad (2)$$

The linearized function through origin with a slope of $S'/X_{\max}$ is expressed as :

$$Y'=\frac{S'}{X_{\max}}\times X \quad (3)$$

where Y' is a converted gray value of each pixel.

Solving the equation (1) and (2) for X and $X_{\max}$, respectively, yields the following equations.

$$X=\left[\frac{1}{\lambda}\times\{1\text{ n } S-1\text{ n}(S-Y)\}\right]^{1/\alpha} \quad (4)$$

$$X_{\max}=\left[\frac{1}{\lambda}\times\{1\text{ n } S-1\text{ n}(S-S')\}\right]^{1/\alpha} \quad (5)$$

The variable X and the constant $X_{\max}$ in equation (3) can now be substituted for equation (4) and (5), respectively, to obtain the following relationship :

Fig. 1 Images of a whole body autoradiogram before (A) and after (B) correcting shading distortions.

After correcting shading distortions, residual distortions, that is, difference of gray value of background area between center and edge of the image was no more than 1% (less than 3 degree in gray value).

Fig. 2 An image of a ROI(A) and the histogram of grayvalues in the ROI(B).

A ROI containing a crack and background field (shown by arrows in the figure) gave a peak in low density area of the histogram.

The X-axis of the histogram indicates gray level and Y-axis indicates a relative amount of each gray level in the ROI.

Fig. 3 A typical calibration curve generated from standard tissues. Data were found to fit a non-linear saturation function described in Materials and methods. Saturation level S was 255, kinetic constant λ was 0.0390774 and correction factor α was 1.02257 respectively.

Fig. 4 A linearized image of whole body autoradiogram of a rat killed 1 hr after administration of radioactive indomethacin. Linearization was performed by the method described in Materials and methods. The color scale in the upper right corner of the figure represents tissue indomethacin concentration in μg equivalent/g tissue. Standard tissue sections (submaxillary gland, pancreas, spleen, liver, kidney muscle and testis) can be seen in the upper part of the figure.

$$Y' = S' \times \left\{ \frac{\ln S - \ln(S - Y)}{\ln S - \ln(S - S')} \right\}^{1/\alpha} \quad (6)$$

and this equation shows the relationship between original (Y) and converted (Y') gray values. Original pixels in an image of whole body autoradiogram were converted to linearly scaled ones using this function. This linearization process is indispensable to obtain radioactivity concentration by extrapolating a mean gray value of a ROI to the calibration curve, because extrapolation of a mean gray value to a non-linear function does not give mean radioactivity concentration when gray values in the ROI vary widely.

(9) Histogram of gray values in a ROI

In order to obtain a mean gray value of a ROI, it is important to check the histogram of gray values in the ROI. As can be seen in Fig. 2, presence of a crack or background field in a ROI causes an appearance of a peak in low density area of the histogram. High density spot such as chemical artifact is often observed in a ROI in a magnified image. In such cases, a peak in high density area is observed in the histogram. These area should be neglected by averaging the gray values which fall into an meaningful range in the histogram of the ROI. The operating software in the present video analysis system enables us to select the range of gray value which contains gray values to be used to calculate the mean gray value. By using this limited averaging method, the effect of cracks or artifact spots can be avoided.

Results and discussion

Fig. 3 shows a calibration curve generated by using eight standard tissues (testis, muscle, spleen, submaxillary gland, pancreas, kidney cortex, kidney medulla and liver from a rat killed 0.5 hr after p.o. administration of radioactive indomethacin). A linearized image of whole body autoradiogram according to the calibration function is shown in Fig. 4. By this linearization, gray value of each pixel was linearly scaled to drug concentration. Therefore, it is possible to estimate drug concentration in tissues by visual inspection.

Distribution of indomethacin in several tissues was determined by using linearized images of whole body autoradiograms, and the result was compared with that obtained by the scintillation counting method (Table I-IV). The results obtained by both methods were almost identical in most of tissues. However, indomethacin concentration in the lung determined by the video analysis method was considerably high as compared with that obtained by scintillation counting method. In the present study, indomethacin concentration in the blood was quite high as compared with that in the lung in both p.o. and i.v. studies. This might be responsible for the difference between drug concentration in the lung determined by video analysis and scintillation counting methods. When animals were killed soon after i.v. administration without bleeding, drug concentration in a capillary rich tissue was higher than that obtained from animals killed by bleeding (our preliminary result). Capillary blood is, therefore, likely to affect the determination of drug concentration by the video analysis method when blood level of a drug is much higher than that in a capillary rich tissue (e.g. lung, liver or kidney). Our preliminary studies also showed that drug concentration in the lung determined by the video analysis method was lower than that determined by the scintillation counting method when blood level of the drug was considerably lower than that of the lung. It was

Table I. Tissue indomethacin concentration determined by video analysis method (VAM) and scintillation counting method (SCM).
10 mg/kg p.o. 1 hr after administration.

Tissue	VAM ($\mu\text{g/g}$ tissue)	SCM ($\mu\text{g/g}$ tissue)	VAM/SCM (%)
Blood	31.64 $\pm$ 1.57	34.81 $\pm$ 5.19	90.89
Cerebrum	0.96 $\pm$ 0.08	0.85 $\pm$ 0.17	112.94
Cerebellum	1.22 $\pm$ 0.10	1.02 $\pm$ 0.23	119.61
Hypophysis	9.72 $\pm$ 1.47	8.46 $\pm$ 1.32	114.89
Spinal cord	1.00 $\pm$ 0.08	0.92 $\pm$ 0.23	108.70
Eye ball	2.61 $\pm$ 0.38	2.46 $\pm$ 0.74	106.10
Harderian gland	7.34 $\pm$ 0.64	7.09 $\pm$ 1.44	103.53
Submaxillary gland	8.54 $\pm$ 0.92	8.45 $\pm$ 1.56	101.06
Thymus	4.24 $\pm$ 0.42	3.63 $\pm$ 0.64	116.80
Heart	9.64 $\pm$ 1.58	10.34 $\pm$ 1.76	93.23
Lung	15.48 $\pm$ 0.64	10.15 $\pm$ 1.40	152.51
Liver	28.34 $\pm$ 0.85	28.02 $\pm$ 3.54	101.14
Kidney cortex	30.18 $\pm$ 1.41	30.06 $\pm$ 5.24	100.40
Kidney medulla	25.49 $\pm$ 0.49	25.95 $\pm$ 5.34	98.23
Spleen	6.38 $\pm$ 0.18	5.33 $\pm$ 0.94	119.70
Pancreas	8.34 $\pm$ 0.97	7.16 $\pm$ 1.36	116.48
Adrenal	12.17 $\pm$ 2.63	10.54 $\pm$ 2.12	115.46
Fat	3.18 $\pm$ 0.34	3.62 $\pm$ 0.74	87.85
Muscle	3.73 $\pm$ 0.12	3.52 $\pm$ 0.59	105.97
Bone marrow	9.45 $\pm$ 1.44	9.78 $\pm$ 2.50	96.63
Testis	3.55 $\pm$ 0.27	3.17 $\pm$ 0.61	111.99
Stomach	13.69 $\pm$ 1.80	15.22 $\pm$ 2.65	89.95
Ileum	7.67 $\pm$ 0.95	6.16 $\pm$ 0.94	124.51
Colon	7.60 $\pm$ 1.22	6.64 $\pm$ 1.20	114.46

Each value represents the mean $\pm$ S.E. (n=3). Correlation coefficient was 0.989.

Table II. Tissue indomethacin concentration determined by video analysis method (VAM) and scintillation counting method (SCM).
10 mg/kg p.o. 2 hr after administration.

Tissue	VAM ($\mu\text{g/g}$ tissue)	SCM ($\mu\text{g/g}$ tissue)	VAM/SCM (%)
Blood	31.43 $\pm$ 1.33	31.56 $\pm$ 1.91	99.59
Cerebrum	0.69 $\pm$ 0.08	0.77 $\pm$ 0.10	89.61
Cerebellum	0.78 $\pm$ 0.01	0.99 $\pm$ 0.12	78.79
Hypophysis	9.65 $\pm$ 0.46	11.80 $\pm$ 2.34	81.78
Spinal cord	0.88 $\pm$ 0.11	0.98 $\pm$ 0.08	89.80
Eye ball	2.43 $\pm$ 0.20	2.12 $\pm$ 0.64	114.62
Harderian gland	6.88 $\pm$ 0.23	6.65 $\pm$ 0.89	103.46
Submaxillary gland	7.49 $\pm$ 0.41	7.50 $\pm$ 0.65	99.87
Thymus	3.66 $\pm$ 0.36	3.78 $\pm$ 0.31	96.83
Heart	10.05 $\pm$ 0.43	9.52 $\pm$ 0.62	105.57
Lung	14.38 $\pm$ 0.55	10.63 $\pm$ 0.63	135.28
Liver	27.23 $\pm$ 1.41	27.84 $\pm$ 0.84	97.81
Kidney cortex	29.75 $\pm$ 1.08	29.40 $\pm$ 2.67	101.19
Kidney medulla	28.40 $\pm$ 0.86	25.45 $\pm$ 1.67	111.59
Spleen	5.45 $\pm$ 0.15	4.83 $\pm$ 0.34	112.84
Pancreas	6.54 $\pm$ 0.78	6.31 $\pm$ 0.58	103.65
Adrenal	7.62 $\pm$ 0.48	7.85 $\pm$ 0.41	97.07
Fat	3.54 $\pm$ 0.29	3.61 $\pm$ 0.42	98.06
Muscle	3.75 $\pm$ 0.15	3.02 $\pm$ 0.15	124.17
Bone marrow	8.65 $\pm$ 0.18	8.90 $\pm$ 0.44	97.19
Testis	5.17 $\pm$ 0.20	5.13 $\pm$ 0.55	100.78
Stomach	7.84 $\pm$ 0.42	7.99 $\pm$ 0.36	98.12
Ileum	24.32 $\pm$ 0.72	39.67 $\pm$ 13.52	61.31
Colon	6.27 $\pm$ 0.69	6.70 $\pm$ 0.99	93.58

Each value represents the mean $\pm$ S.E. (n=3). Correlation coefficient was 0.954.

Table III. Tissue indomethacin concentration determined by video analysis method (VAM) and scintillation counting method (SCM).
1 mg/kg i.v. 15 min after injection.

Tissue	VAM ($\mu\text{g/g}$ tissue)	SCM ($\mu\text{g/g}$ tissue)	VAM/SCM (%)
Blood	4.42 ± 0.10	4.10 ± 0.21	107.80
Cerebrum	0.11 ± 0.01	0.10 ± 0.01	110.00
Cerebellum	0.99 ± 0.01	0.11 ± 0.00	81.82
Hypophysis	0.99 ± 0.10	1.16 ± 0.26	85.34
Spinal cord	0.11 ± 0.05	0.10 ± 0.02	110.00
Eye ball	0.17 ± 0.02	0.16 ± 0.02	106.25
Harderian gland	0.67 ± 0.05	0.71 ± 0.03	94.37
Submaxillary gland	0.97 ± 0.01	0.95 ± 0.04	102.11
Thymus	0.37 ± 0.02	0.37 ± 0.02	100.00
Heart	1.31 ± 0.06	1.21 ± 0.08	108.26
Lung	1.72 ± 0.16	1.42 ± 0.08	121.13
Liver	3.39 ± 0.15	3.36 ± 0.04	100.89
Kidney cortex	5.46 ± 0.32	5.01 ± 0.22	108.98
Kidney medulla	4.68 ± 0.37	4.58 ± 0.28	102.18
Spleen	N. D.	0.66 ± 0.01	—
Pancreas	0.68 ± 0.09	0.76 ± 0.02	89.47
Adrenal	1.16 ± 0.12	1.12 ± 0.02	103.57
Fat	0.38 ± 0.06	0.32 ± 0.02	118.75
Muscle	0.38 ± 0.04	0.41 ± 0.01	92.68
Bone marrow	1.08 ± 0.03	1.00 ± 0.02	108.00
Testis	0.22 ± 0.02	0.18 ± 0.01	122.22
Stomach	0.61 ± 0.05	0.74 ± 0.03	82.43
Ileum	0.62 ± 0.08	0.68 ± 0.05	91.18
Colon	0.61 ± 0.05	0.60 ± 0.04	101.67

Each value represents the mean $\pm$ S.E. (n=3). Correlation coefficient was 0.998.

N.D. means "not detected" because of the lack of a suitable area in the autoradiograms.

Table IV. Tissue indomethacin concentration determined by video analysis method (VAM) and scintillation counting method (SCM).
1 mg/kg i. v. 6 hr after injection.

Tissue	VAM ($\mu\text{g/g}$ tissue)	SCM ($\mu\text{g/g}$ tissue)	VAM/SCM (%)
Blood	1.07 ± 0.01	0.98 ± 0.13	109.18
Cerebrum	0.03 ± 0.01	0.03 ± 0.01	100.00
Cerebellum	0.03 ± 0.01	0.03 ± 0.00	100.00
Hypophysis	0.26 ± 0.04	0.24 ± 0.04	108.33
Spinal cord	0.03 ± 0.01	0.04 ± 0.01	75.00
Eye ball	0.06 ± 0.01	0.06 ± 0.01	100.00
Harderian gland	0.22 ± 0.02	0.20 ± 0.03	110.00
Submaxillary gland	0.21 ± 0.02	0.19 ± 0.03	110.53
Thymus	0.10 ± 0.01	0.10 ± 0.02	100.00
Heart	0.26 ± 0.00	0.28 ± 0.04	92.86
Lung	0.44 ± 0.02	0.36 ± 0.05	122.22
Liver	1.12 ± 0.08	1.24 ± 0.06	90.32
Kidney cortex	1.69 ± 0.00	1.51 ± 0.14	111.92
Kidney medulla	1.33 ± 0.08	1.37 ± 0.09	97.08
Spleen	N. D.	0.16 ± 0.03	—
Pancreas	0.18 ± 0.01	0.17 ± 0.02	105.88
Adrenal	0.26 ± 0.03	0.23 ± 0.04	113.04
Fat	0.10 ± 0.03	0.09 ± 0.01	111.11
Muscle	0.10 ± 0.04	0.09 ± 0.02	111.11
Bone marrow	N. D.	0.24 ± 0.03	—
Testis	0.25 ± 0.02	0.22 ± 0.02	113.64
Stomach	0.16 ± 0.01	0.17 ± 0.03	94.12
Ileum	2.17 ± 0.43	2.65 ± 0.22	81.89
Colon	0.80 ± 0.09	0.72 ± 0.24	111.11

Each value represents the mean $\pm$ S.E. (n=3). Correlation coefficient was 0.987.

N.D. means "not detected" because of the lack of a suitable area in the autoradiograms.

assumed that lower specific gravity of the lung due to the presence of alveoli was responsible for giving a lower drug concentration. Therefore, it is required to check the effects of blood level of a drug and density of the lung on the determination of drug concentration in the lung by the video analysis method. In the present study, indomethacin concentration in blood was almost the same as that in the liver or kidney. This might be the reason why the result of the video analysis method was similar to that obtained by the scintillation counting method in determining indomethacin concentration in the liver and kidney.

Indomethacin concentration in the ileum from rats killed 2 hr after p.o. administration was determined as $24.32 \pm 0.72 \mu\text{g/g}$ (mean $\pm$ S.E.) by the video analysis method, and the value was considerably lower than that obtained by the scintillation counting method. However, the scintillation counting method gave the values of 24.87, 66.66 and $27.48 \mu\text{g/g}$ for each of three ileum samples giving a mean value of $39.67 \pm 13.52 \mu\text{g/g}$. It was supposed that intestinal contents containing high radioactivity remained in one of the ileum samples.

Fig. 5 shows the relationship between video analysis and scintillation counting methods in determining tissue indomethacin concentration in both p.o. and i.v. experiments (except for data from all lung samples and ileum sample obtained 2 hr after p.o. administration).

Fig. 5. Relationship between video analysis and scintillation counting methods in determining tissue indomethacin concentration.

Data were obtained from both p.o. and i.v. experiments (10 mg/kg p.o. 1 and 2 hr after administration and 1 mg/kg i.v. 15 min and 6 hr after injection) except for all lung samples and ileum sample obtained from rats killed 2 hr after p.o. administration.

There was a linear relationship which was expressed by the following equation :

$$Cva = 0.989324 \times Csc + 0.121247$$

where Cva is a value obtained by the video analysis method and Csc is that obtained by the scintillation counting method. The correlation coefficient was 0.997. Our preliminary studies using mice, monkeys and female rats also showed that the video analysis method was agreed well with the scintillation counting method in determining drug concentration in tissues. This must be confirmed in different kind of drugs, different routes of administration and different ages of animals. Because this video analysis method has an advantage as compared with the scintillation counting analysis of separated tissue, i.e. high resolution is easily achieved to analyze a discrete region which is difficult to separate anatomically. For instance, large tissues of fetal rat can be analyzed from 13 days after pregnancy and almost all tissues can be identified in a fetus 18 days after pregnancy. This method will, therefore, be valuable in not only drug distribution studies but also toxicity studies concerning drug teratogenicity.

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