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A STUDY ON THE MEASUREMENT OF THE VOLUME OF THE CIRCULATORY BLOOD OF THE HORSE

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A survey on the methods of measurement of circulatory blood volume in the living body was made. As a direct method it was noted that HARVEY deduced the blood volume from the loss in body weight following bleeding to death while WELEKER is reported to have used living body perfusion methods.[22] As an indirect method for measurement, substances with a special affinity to either blood corpuscles or plasma are used. As other indirect methods, dye methods which have to do with plasma capacity and a CO method which has to do with blood corpuscle capacity, have been hitherto utilized. However, in place of the CO method, radio isotopes such as[19,21,23,24] ^{59}Fe, ^{32}P are now being used to measure blood corpuscle volume.

KEITH et al. introduced circulatory blood volume measurement by the dye method by advocating the use of vitalred. A little later DAWSON et al. reported that the blue dye T-1824 was most suitable for the measurement of circulatory blood volume. Later, GIBSON & EVANS, using T-1824 standardized the measurement of circulatory blood volume. Still later GREGERSEN[11,12] and a co-worker made clinical adaptations and finally produced a simplified measurement method. Today the EVANS blue method is the most widely used among dye methods. GIBSON & EVANS found that a chronological follow-up on dye concentration in blood, after infusion of dye, showed a rapid lowering of dye concentration in the initial stage forming a mixing curve while this stage was followed by a gradual and constant lowering which formed a disappearance slope. From this result, GIBSON & EVANS believed that the straight section of the curve could be extrapolated to the minute line and thus theoretically it was possible to calculate the dye concentration in blood prior to the time of disappearance of the dye therefrom and at the exact time when a complete mixing took place. Thus, measurement of circulatory blood volume by so-called extrapolation methods was standardized. This in essence, is the EVANS blue method used hitherto. In contrast to the above, GREGERSEN discovered that the value of the dye concentration in blood 10 minutes after infusion of the dye generally coincided with the EVANS blue method values

and invented a simplified method based on measurement of dye concentration in minute plasma after infusion of dye. However, as a result of work by DAWSON et al., the following was revealed. According to his report, a follow-up on dye concentration in blood after dye infusion in dog, revealed that up till the first hour a regular exponential decrease (rapid disappearance phase) was shown which was followed by an extremely slow, constant, straight line decrease in dye concentration (exponential phase). Therefore, when dyeless plasma is infused in this period and the degree of dilution of dye is measured, circulatory plasma volume can be calculated. LAWSON[17,18,20] called this method the "dye-decrement method." Now, the plasma volume thus obtained showed an approximate 15% decrease in value as compared with GIBSON & EVANS extrapolation method. LAWSON explained that this is due to the fact that when T-1824 is infused into the blood vessel, part of the dye immediately adheres to the blood vessel walls which cause a decrease in the dye concentration in blood. Thus, in methods employed hitherto in which no allowance is made for the above, it is only natural that an overestimation in plasma volume resulted. Further, LAWSON reported that in the rapid disappearance phase, since the logarithm of dye concentration forms a straight line as compared with the logarithm of time (minutes), the dye concentration value is obtained by extrapolating this straight line to 0 minute of the time line; in other words, the values coincide with values obtained by the dye-decrement method.

Thus, with the recent advancement in studies on circulatory blood volume measurements, various reports have been made by various means in the rabbit, dog and man. However, it is regrettable that insofar as the horse is concerned no reliable values can be found. As to existing literature only 2 or 3 reports are to be seen. From literature at hand, REICHERT & BROWN using WELEKER'S living body perfusion method reported that total blood volume is 9.7% of body weight; COURTICE using 2 horses made measurements by EVANS blue method and reported that plasma volume and total blood volume per kg body weight were 51 ml, 72 ml respectively; CRONIN using a single horse made measurements by EVANS blue method and reported 52.37 ml, 81.14 ml per kg body weight respectively in plasma volume and blood volume. As shown here not only are the reports on circulatory blood volume measurements in horse limited but also the number of horses used has been limited to 1 or 2. In spite of the adove, the author is of the opinion that accurate measurements on circulatory blood volume in horse and knowledge related to measured values would be of great importance in studies using horses and also in clinical extension work. Nowadays, it is generally accepted that the radioisotope method is valuable in supplementing dye methods and that the values obtained are higher in accuracy.

This radioisotope label method consists of labeling red blood corpuscles with radio elements. Radiophosphorous (^{32}P),[17] radio-iron ($^{55,59}Fe$),[13] radio chrome (^{51}Cr),[16] radiopotassium (^{42}K)[1,25] etc. are used. Among these radioactive substances ^{32}P is considered to be most useful owing to its availability, price, time of degeneration, and lack of danger because of its having β-radiation. However, at present there are very few reports[8] available on circulatory blood volume measurements using radioisotope labeling in domestic animals. Accordingly, the author has made the herein described attempt to obtain values on circulatory blood volume measurement in horse using ^{32}P labeling.

For the above reasons, the present studies were carried out with the focus of attention on re-evaluation of the dye method and radioisotope labeling method and comparisons of results measured, with the intent of standardizing a practical, readily adaptable method for measurement of circulatory blood volume in the horse. At the same time circulatory blood volume measurements of a large number of healthy horses, using the above methods, were carried out.

EXPERIMENTAL METHODS

I. Measurement of circulatory plasma volume and circulatory blood volume by T-1824

Circulatory plasma volume measurement

In this experiment 32 healthy horses were used. In 28 cases, 0.5% EVANS blue solution was administered through the jugular vein on one side at a rate of 0.20~0.586 mg/kg. As a precautionary step so that no dye would remain in the syringe, blood was drawn into the syringe 3 times to flush out any remaining dye. Syringes of the same size were used; the syringe capacity was precisely adjusted. Indexed syringes with 50 ml marking were used. Blood samples were collected at 2, 5, 10 and 15 minutes up till one hour and a half after dye infusion. The samples were left standing at room temperature for 24 hours after which plasma was separated. In addition this material was centrifuged in order to insure complete separation of plasma. The plasma thus obtained was investigated electro-colorimetrically, dyeless plasma separated from blood samples taken prior to dye infusion being used as control. The respective transmissivities were checked against standard tables and extinction was calculated. As to the electrocolorimeter, ELMAR's Model 2 was used. The fluid tank was 10 mm thick and the filter used was S-61.

The dye concentration at each point thus estimated was charted as follows. The time (minutes) was indicated on the horizontal axis while the extinction was indicated on the vertical axis. By GIBSON & EVANS' extrapolation method, the maximum value of the extinction of even dye concentration in blood was obtained. Further, the above was re-indicated in a logarithm graph of both axis in which the logarithm of time (in minutes) was indicated on the horizontal axis and the logarithm of 10 times the extinction was indicated on the vertical axis. The one minute value of the extinction was calculated by LAWSON's logarithmic extrapolation. Errors coming from extrapolation were minimized by the least

square method. Next, prior to the calculations of circulatory plasma volume, the correlation between T-1824 and the colorimeter was measured. This was done in the following manner. Dye columns of various concentrations within the limits of the experiments were made, using plasma; the respective extinctions were calculated from plasma in which dye concentration is known. According to LAMBERT-BEER's Law since $E=KC$ (E=extinction, C=concentration, K=constant), K is calculated from each diluted solution and the mean value of the resulting K's was determined by which to calculate the plasma volume. C was expressed by the dilution rate against 0.5% EVANS blue original. Thus the circulatory plasma volume was obtained by the following formula: $PV_{T\text{-}1824}=\frac{KC}{D}$ where $PV_{T\text{-}1824}$ is the plasma volume to be obtained (ml), and K determined as described above is constant. C expresses the infused dye volume (ml) and D stands for extinction calculated from GIBSON & EVANS' 0 minute value, GREGERSEN's 10 minute value and LAWSON's one minute value. Using plasma volume thus obtained and the hematocrit (Ht) value measured prior to dye infusion, circulatory blood volume was calculated by the formula $BV=\frac{PV_{T\text{-}1824}\times 100}{100-Ht\times 0.96}$, where BV is the circulatory blood volume to be obtained (ml) and $PV_{T\text{-}1824}$ is the circulatory plasma volume (ml). Ht values were measured by making a flat bottomed tube with a capacity approximately similar to a Sahli's colorimeter. As an anticoagulant 0.1 ml of ammonium and potassium oxalates solution was added. The material was centrifuged at 3,000 r. p. m. for 30 minutes and after deduction of the anticoagulant volume, Ht values were obtained. The figure 0.96 comes from GREGERSEN's adapted value and is based on the idea that approximately 4% of blood plasma still remains in the centrifuged corpuscle volume.

II. Measurement of circulatory plasma volume and blood volume by the use of ^{32}P

The radioisotope used in the present studies was obtained from "The Radiochemical Centre", (Amersham, England) through the Japan Radioisotope Association (Science Institute of Japan). The original stock is dissolved in diluted hydrochloric acid as a carrier free solution (pH 2～3). Prior to use, this stock was neutralized with 0.01 N NaOH to which physiological saline solution was added to maintain the radioactivity of ^{32}P at approximately 20 μc/ml.

Method of making ^{32}P labeled red corpuscles

In all cases syringes containing 2 ml of 10% Na-citrate were used to collect 18 ml of blood samples from the jugular vein of experimental animals; part of the samples was set aside for Ht while the remainder was transferred into indexed centrifuge tubes and centrifugally precipitated for 30 minutes at 3,000 r.p.m. The plasma was discarded, and 250 μc ^{32}P containing physiological saline solution was added.

Next this material was heated at 37°C for 2 hours, and shaken at 20 minute intervals, thus labeling the red blood corpuscles. This preparate was centrifuged and washed in physiological saline (until all radioactivity in the washing had disappeared) after which labeled red blood corpuscles were suspended in physiological saline solution. This suspension fluid was made in equal volume to the initial blood volume.

Procedure for measurement of circulatory blood volume

Exactly 10 ml of the above labeled suspension fluid was injected into the left jugular vein of the experimental animals using a certified syringe (it is important to check whether the hypodermic needle is inserted into the blood vessel before infusion, then after infusion, the blood is drawn into the syringe 2~3 times to flush out remaining labeled corpuscles). At 5, 10, 15, 20, 30, 40, 50, 60, 90 and 120 minutes after the injection, blood samples were taken from the opposite jugular vein.

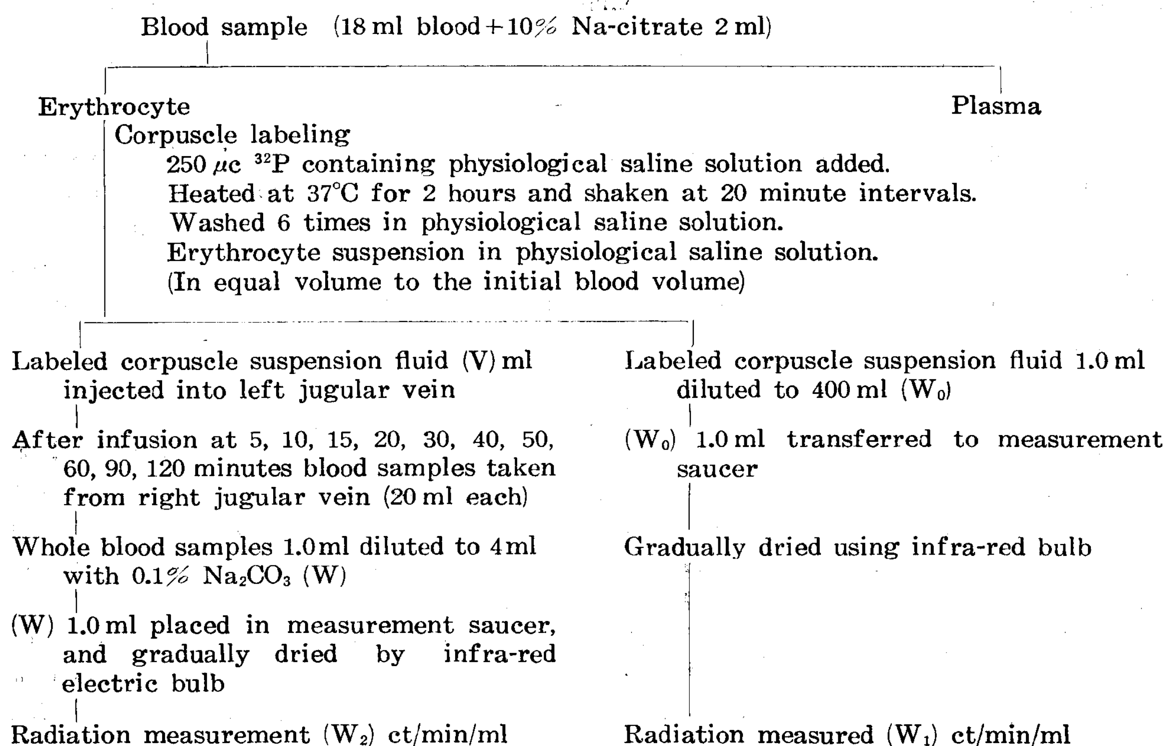
Procedure for making radioactive measurement samples

The two methods described below were used to make samples for measurement.

a) Method using whole blood (Table 1)

First, 2 ml portions of 0.1% Na_2CO_3 each were placed in separate test tubes to which 1 ml of each blood sample was added in order to induce complete hemolysis. From each tube 1 ml was placed in a metal measurement saucer (stainless steel, round 15 mm in diameter and 7 mm deep); by use of an infra-red electric bulb the samples were gradually dried in such a way that the residue adhered evenly to the bottom of the saucer. In order to determine the intensity of the infused radioactivity, as control, a similar sample was

TABLE 1. *Procedure for Measurement of Circulatory Blood Volume* (Method using whole Blood)



Note: (W_2) indicates the value obtained by extrapolating the extinction curve to zero.

diluted 400 times with 0.1% red corpuscle suspension solution and 1 ml of this was dried in the same manner as above. By the use of this method, calcination can be dispensed with and volume of circulatory blood can be calculated directly. However, since the self absorption of radiation of solids in blood is high, correction of the values was made as will be described in later experiments.

b) Method using red corpuscles

In order to calculate labeled corpuscle radioactivity, as control, 1 ml of labeled corpuscle suspension solution was placed in a Sahli tube. This was centrifuged and the corpuscle volume was calculated by reading the index of the blood corpuscle column. Next, plasma was removed from the above and distilled water was added to the remainder; preparate was hemolysed. This hemolysed red blood corpuscle material was washed out into a Kjeldahl's microflask. In order to determine the radioactivity of blood samples taken, 1 ml exactly of each blood sample taken at designated intervals was placed in test tubes and left standing. After coagulation the serum was discarded and the remainder was washed out into Kjeldahl's microflasks. Besides this, a given amount of Na-citrate was pipetted into Sahli tubes to which blood samples were added. Then the red blood corpuscles were calculated and the values were considered to represent the volume of the red blood corpuscles in coagulum in the test tubes. The control and labeled corpuscles at each interval were transferred to Kjeldahl's microflasks and concentrated nitric acid, concentrated sulfuric acid and perchloric acid were added. This was heated and wet calcinated. When the material was completely calcinated, primary sodium phosphate was added to which a mixed solution of ammonia was added to bring about a co-precipitation of phosphorus. This was left standing for 24 hours. Next, the precipitate of magnesium phosphate and ammonia phosphate containing ^{32}P, was caused to deposit evenly on filter paper, cut to the same size as the measurement saucer. The paper was placed on the measurement saucer and left standing until dried at room temperature after which the radioactivity was measured.

Procedure for measuring radioactivity

For measurements, Lauritsen's Counter (Made at the Kagaku Kenkyu-jo), Geiger Müller Counter and scaler (Kagaku Kenkyu-jo Model 32) were used Since in the apparatus used here it is known that the measured value errors do not exceed experimental errors, in the present studies the measurement values of both were considered the same. Background measurements were made 1 hour or more before and after sample measurements were made. Since measurement saucers were all made of stainless steel, corrections for backscattering were unnecessary. Further, in regard to error in values coming from physical deterioration of ^{32}P, it was noted that at 6 hours there was an approximate 9% difference. However, since 1 series of measurement did not require more than 3 hours, such errors were disregarded.

Method of calculation

Circulatory blood volume, blood corpuscle volume and plasma volume were calculated in the following manner from radioactivity and extinction.

a) Measurements based on total blood volume

Circulatory blood volume (CBV ^{32}P) by ^{32}P

$$= \frac{\text{Radioactivity in labeled suspension (Wi) ct/min/ml} \times \text{infused volume (V) ml}}{\text{Radioactivity of blood sample (Ws) ct/min/ml}}$$

where the value of infused labeled corpuscle activity is the corrected value obtained by multiplying it by Cr (Table 23, correction value) in order to correct self absorption of radiation. In other words, taking the number of times diluted and correction values into consideration (Table 2)

$$(\text{Wi}) = 400 \times (\text{W}) \times (\text{Cr}); \quad (\text{Ws}) = 4 \times (\text{W}_2)$$

where (Wi) is the infused radioactivity measurement value and (W_2) in the extrapolated value of blood sample radioactivity.

$$\text{Circulatory corpuscle volume (RCV cal. } ^{32}\text{P}) = \frac{\text{CBV } ^{32}\text{P} \times \text{Ht} \times \text{Correction value}}{100}.$$

When Ht values are measured, in order to make allowance for trapped plasma in the blood corpuscle column, a fairly large discrepancy in value should be tolerated.

In the case of human beings, GREGERSEN reported 0.96 ; however in the present studies corrections were made by means of values measured by JENNINGS ^{131}I.[4,14]

$$\text{Plasma Volume (PV cal. } ^{32}\text{P}) = \text{CBV } ^{32}\text{P} - \text{RCV cal. } ^{32}\text{P}$$

TABLE 2. *Measurement of Radio Activity*

*DEGREE OF DILUTION	SAMPLES	OBSERVED VALUE OF THEORETICAL VALUE (Ct/min/ml)	THEORETICAL VALUE (Ct/min/ml)	CORRECTION VALUE
×4	4	6931.0 ± 18.6	6931.0	1
×40	4	785.0 ± 7.5	693.1	0.882
×400	4	80.4 ± 1.3	69.3	0.861

* Degree of dilution of the labeled suspension of which the Ht value has been made about 40% by addition of blood plasma.

b) Measurements made by using red blood corpuscles

Circulatory corpuscle volume (RCV ^{32}P)

$$= \frac{\text{Infused labeled corpuscle radioactivity } (R_1) \text{ ct/min/ml infused labeled corpuscle volume (VR)ml}}{\text{Labeled corpuscle radioactivity of blood samples } (R_2) \text{ ct/min/ml}}$$

$$\text{Circulatory blood volume } (\text{PV}_{\text{T-1824}}) = \frac{\text{PV}_{\text{T-1824}}}{100\text{-Ht} \times \text{correction value}}$$

EXPERIMENTAL RESULTS

1) Comparison of 3 values in T-1824, obtained by LAWSON's, GREGERSEN's and GIBSON and EVANS' methods

Fifteen horses were used. Their body weight, sex, age and volume of T-1824 infused are as exhibited in table 3. In the present experiments T-1824 volume was limited to 0.202~

TABLE 3. *Horses Used in the Experiment and Injected Dose of T-1824* (mg/kg body weight)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY * WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	Ht (%)
Hatsuisamu	Percheron	♂	9	610	0.204	37.0
Wakaba	″	♀	9	690	0.289	33.0
Hatsuhime 1	″	″	7	540	0.231	37.0
Hatsuhime	″	″	3	490	0.204	36.0
Yamazakura	″	″	8	590	0.211	38.0
Hanahime	″	″	2	430	0.232	33.0
Elizabeth	Norman	″	4	445	0.202	36.0
Shirakawa	″	″	14	462	0.216	36.0
Hokuto	″	♂	14	410	0.219	39.0
Appuderu	″	″	10	438	0.205	41.0
Tokushima	″	″	10	420	0.214	42.0
Yoshitaka	″	″	12	450	0.214	34.0
Seijiu	Percheron	″	5	310	0.222	37.5
Kooun	″	♀	4	550	0.218	39.0
Riun	″	″	12	630	0.248	40.2
Average				497	0.207	37.0

* At every time of the experiments, the body weight was weighted respectively.

0.289 mg per kg body weight. Extrapolative methods developed by GIBSON & EVANS and by LAWSON are shown in figs. 1 and 2. According to LAWSON's method, as may be seen in the figure when the logarithm of 10× serum extinction is expressed in the vertical axis, and the logarithm of time (minutes) is expressed in the horizontal axis, the resulting dots form an approximate straight line. From this, when 1 minute values are extrapolated by point selection method, subjective discrepancies may creep into a large extent. Thus, in the present experiments, an appropriate linear experimental formula was obtained by least square method and from this the extinction at 1 minute after dye infusion was calculated. The values thus obtained by the methods are shown in table 4. As may be seen in table 5 both the circulatory blood volume and circulatory plasma volume showed the highest values as obtained by GIBSON and EVANS' method, with intermediate values obtaind by GREGERSON's method and the lowest values by LAWSON's method. When those values are converted and expressed in terms of per kg body weight the results are as in tables 6 and 7. The mean values of the above obtained by the three methods are as shown in fig. 3. As may be seen, circulatory blood volume by LAWSON's method was 62.0 ml, by GREGERSEN's method 72.2 ml and by GIBSON and EVANS' method 76.0 ml while circulatory plasma volume by LAWSON's method was 40 ml, by GREGERSEN's method 46.4 ml and by GIBSON & Evans' method 49.0 ml. Thus it was shown that in terms of per kg body weight

TABLE 4. *Blood and Plasma Volume Determined by the Three Methods of LAWSON, GREGERSEN and GIBSON & EVANS*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME					CIRCULATORY PLASMA VOLUME				
	LAWSON'S method (L)	GREGERSEN'S method (L)	GREGERSEN-LAWSON (%)	GIBSON & EVANS' method (L)	GIBSON & EVANS-LAWSON (%)	LAWSON'S method (L)	GREGERSEN'S method (L)	GREGERSEN-LAWSON (%)	GIBSON & EVANS' method (L)	GIBSON & EVANS-LAWSON (%)
Hatsuisamu	46.6	53.4	14.6	51.3	10.0	30.0	34.5	15.0	33.3	11.0
Wakaba	45.4	51.9	14.3	55.9	23.2	31.0	35.4	14.2	38.2	23.2
Hatsuhime 1	26.2	29.8	13.7	31.8	21.4	16.9	19.3	14.2	20.5	21.3
Hatsuhime	33.5	37.8	13.1	37.9	13.1	21.9	24.8	13.2	24.8	13.2
Yamazakura	33.3	38.6	15.8	40.7	22.1	21.2	24.5	15.5	25.9	22.2
Hanahime	30.5	35.2	15.4	36.4	19.3	20.8	24.1	15.9	24.9	19.7
Elizabeth	21.5	25.8	20.0	27.4	27.4	14.1	16.9	19.8	17.9	26.9
Shirakawa	25.8	28.8	11.6	37.0	43.4	17.0	18.8	10.6	24.2	42.4
Hokuto	27.5	31.0	12.7	32.1	16.7	17.2	19.4	12.8	20.0	16.3
Appuderu	27.1	30.1	21.8	31.0	33.5	16.5	18.2	10.3	18.8	13.9
Tokushima	23.9	29.1	31.1	31.9	46.2	14.3	17.4	21.6	19.1	33.6
Yoshitaka	27.3	35.8	31.1	39.9	46.2	18.4	24.1	31.0	26.9	46.2
Seijiu	19.1	24.3	27.2	25.0	30.9	12.2	15.6	27.8	16.0	31.1
Kooun	33.9	39.1	15.3	39.5	16.5	21.2	24.5	15.6	24.7	16.5
Riun	42.5	49.1	16.1	49.1	16.1	26.0	30.2	16.2	30.2	16.2
Avérage	30.9	35.9	18.3	37.7	25.7	19.9	23.2	16.9	24.3	23.6

in both circulatory blood volume and circulatory plasma volume, values were larger in the order of those obtained by LAWSON's method, GREGERSEN's method and GIBSON & EVANS' method.

TABLE 5. *Blood and Plasma Volume Per kg Body Weight Determined by the Three Methods*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME			CIRCULATORY PLASMA VOLUME		
	LAWSON (ml/kg)	GREGERSEN (ml/kg)	GIBSON & EVANS (ml/kg)	LAWSON (ml/kg)	GREGERSEN (ml/kg)	GIBSON & EVANS (ml/kg)
Hatsuisamu	76.4	87.5	84.0	49.1	56.5	54.5
Wakaba	65.8	75.2	82.5	44.9	51.3	55.4
Hatsuhime 1	48.5	55.2	58.8	31.3	35.7	37.9
Hatsuhime	68.4	77.3	77.4	44.7	50.6	50.7
Yamazakura	56.4	65.4	69.0	35.9	41.5	43.8
Hanahime	70.9	81.9	84.5	48.4	56.0	58.9
Elizabeth	48.3	57.9	61.6	31.7	37.9	40.3
Shirakawa	55.8	62.3	80.1	36.8	40.7	52.4
Hokuto	67.0	75.6	78.2	41.9	47.3	48.9
Appuderu	61.9	68.7	70.8	37.7	41.5	42.9
Tokushima	56.9	69.3	76.1	34.0	41.4	45.4
Yoshitaka	60.7	79.6	88.7	40.9	53.5	59.5
Seijiu	61.6	78.4	80.6	39.3	50.3	51.6
Kooun	61.6	71.0	71.8	38.5	44.5	44.9
Riun	67.1	77.9	77.9	41.3	47.9	47.9
Average	62.0	72.2	76.0	40.0	46.4	49.0

FIG. 1. *Extrapolation of GIBSON & EVANS' Method*

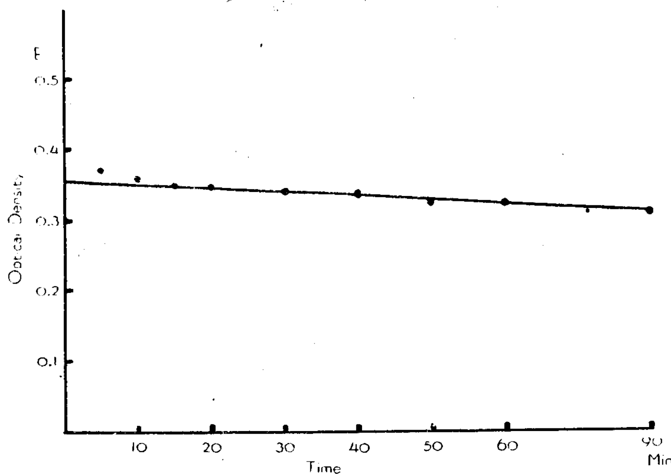


FIG. 2. *Extrapolatian of LAWSON's Method*

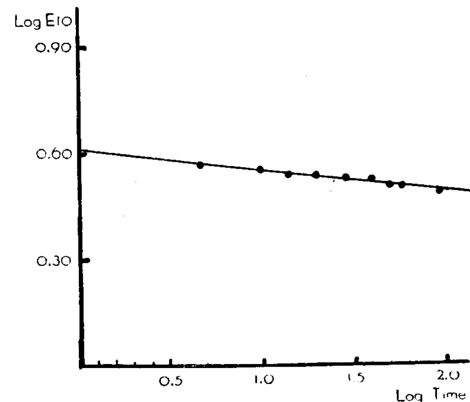


TABLE 6. *Horses Used in Experiment and Injected Dose of T-1824* (0.202～0.214 mg/kg)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	Ht (%)
Hatsuisamu	Percheron	♂	9	610	0.204	37.0
Hatsuhime	〃	♀	3	490	0.204	36.0
Elizabeth	Norman	〃	4	445	0.202	36.0
Appuderu	〃	♂	10	438	0.205	41.0
Yamazakura	Percheron	♀	8	590	0.211	38.0
Tokushima	Norman	♂	10	420	0.214	42.0
Yoshitaka	〃	〃	12	450	0.214	34.0

TABLE 7. *Blood Volume and Plasma Volume Determined by LAWSON's Method*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME		CIRCULATORY PLASMA VOLUME	
	(L)	(ml/kg)	(L)	(ml/kg)
Hatsuisamu	46.6	76.4	30.0	49.1
Hatsuhime	33.5	68.4	21.9	44.7
Elizabeth	21.5	48.3	14.1	31.7
Appuderu	27.1	61.9	16.5	37.7
Yamazakura	33.3	56.4	21.2	44.7
Tokushima	23.9	56.9	14.3	34.0
Yoshitaka	27.3	60.7	18.4	40.9
Average	30.47	61.14	19.48	40.40

TABLE 8. *Horses Used in the Experiment and Injected Dose of T-1824* (0.216～0.248 mg/kg)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	Ht (%)
Shirakawa	Norman	♀	14	462	0.216	36.0
Hokuto	〃	♂	14	410	0.219	39.0
Kooun	Percheron	♀	4	550	0.218	39.0
Hatsuhime 1	〃	〃	7	540	0.231	37.0
Hanahime	〃	〃	2	430	0.232	33.0
Seijiu	〃	♂	5	310	0.222	37.5
Riun	〃	♀	12	630	0.248	40.2

FIG. 3. *Blood and Plasma Volume Determined by the Three Methods*

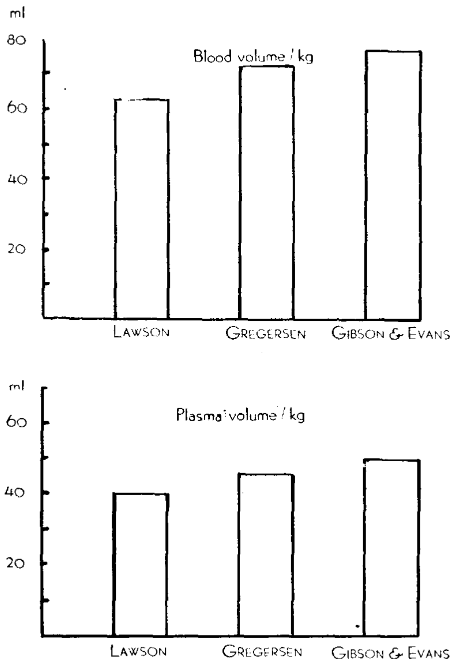


FIG. 4. *Blood and Plasma Volume Determined by the Three Methods*

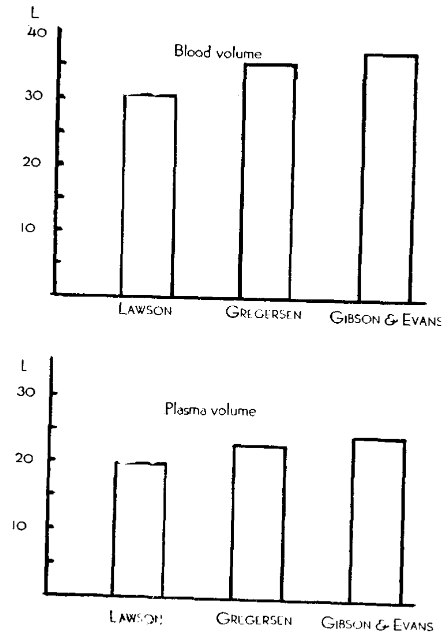


FIG. 5. *Relationship Between Blood Volume and Injected Dye Dose*

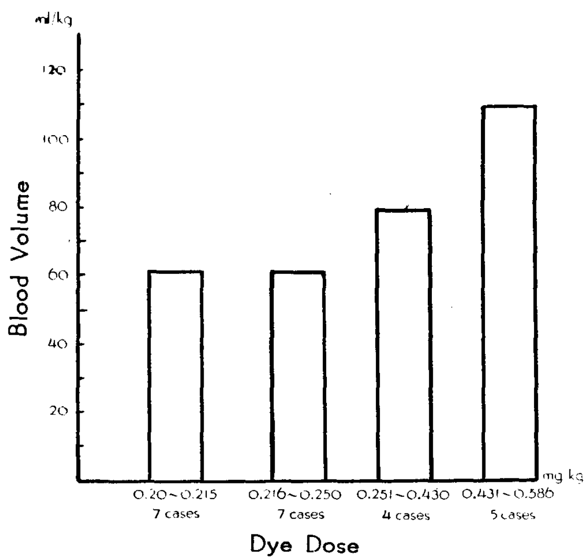
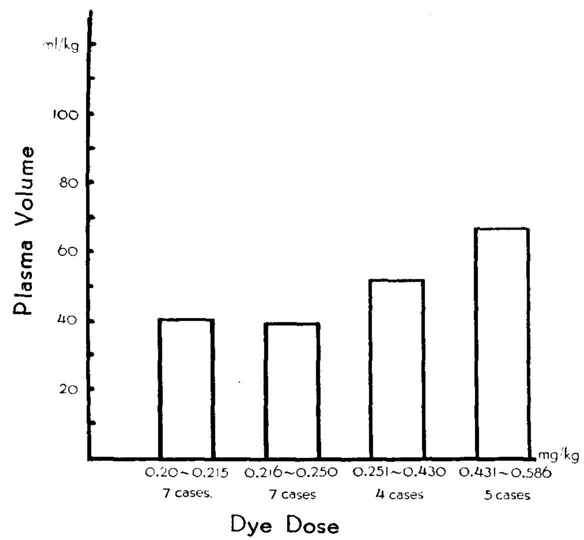


FIG. 6. *Relationship Between Plasma Volume and Injected Dye Dose*



2) Differences by volume of infused T-1824

T-1824 which combines with plasma albumin, is considered as a comparatively hard-to-eliminate dye, owing to the fact that it adheres to blood vessel walls and partially disappears from the blood stream which fact was pointed out by LAWSON et al.[18] Hence, it may be expected that the amount of dye adhering to the blood vessel walls would be influenced by the volume of infused T-1824. Accordingly, a comparative study was made on circulatory blood volume and circulatory plasma volume by varying the volume of infused dye. Twenty-three experimental horses were divided into 4 groups in the following manner: per kg body weight T-1824 infusion volume 0.202~0.214 mg (7 cases), 0.216~0.248 mg (7 cases), 0.289~0.430 mg (4 cases), 0.431~0.586 mg (5 cases). The individual horses and their respective body weights, ages and the infused volumes are indicated respectively in tables 6, 8, 10 and 12. The circulatory blood volume and circulatory plasma volume per kg body weight are indicated respectively in tables 8~13. With regard to their mean values as seen infigs. 5, 6 in all 3 methods within the range of 0.200~0.250 mg/kg, no significant difference was observed between the circulatory blood volume and circulatory plasma volume.

However, when the volume of dye was increased, both circulatory blood volume and circulatory plasma volume showed high values. The correlation between circulatory blood volume and circulatory plasma volume per kg body weight as calculated from infused dye volume; the 3 methods are shown in figs. 7~12. As shown, the correlation coefficient, in all cases was significant at 0.1 critical rate. Therefore, generally, when the infused dye volume increases, it may be said that high values are to be expected in both the circulatory blood volume and circulatory plasma volume. However, as may be seen in figs. 3, 4, within the range of 0.200~0.250 mg infused dye volume per kg body weight since no significant difference can be seen, in the case of measurement of circulatory blood volume by T-1824, the appropriate volume range for practical purposes would be 0.200~0.250 mg.

FIG. 7. *Correlation Between Plasma Volume Per kg Body Weight Determined by GIBSON & EVANS' Method and Injected Dye Dose*

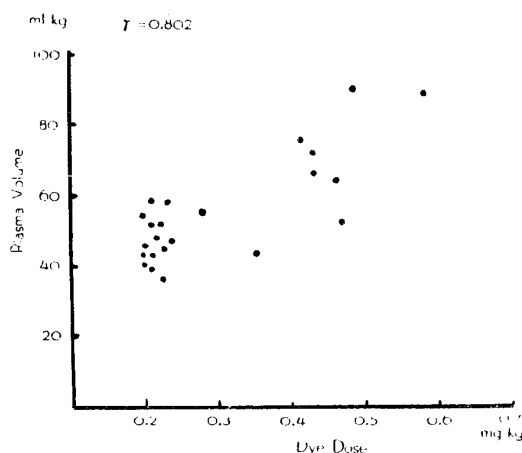


FIG. 8. *Correlation Between Plasma Volume Per kg Body Weight Determined by GREGERSEN's Method and Injected Dye Dose*

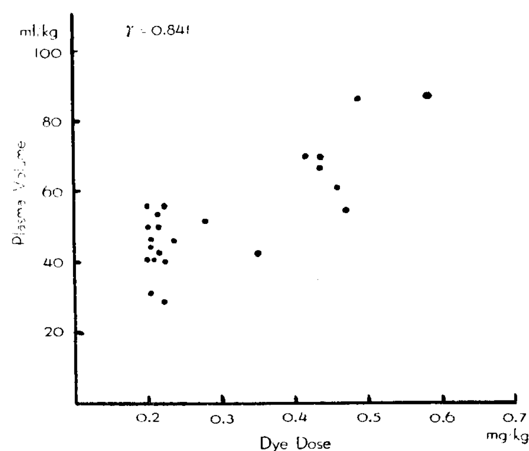


FIG. 9. *Correlation Between Plasma Volume Per kg Body Weight Determined by* LAWSON'S *Method and Injected Dye Dose*

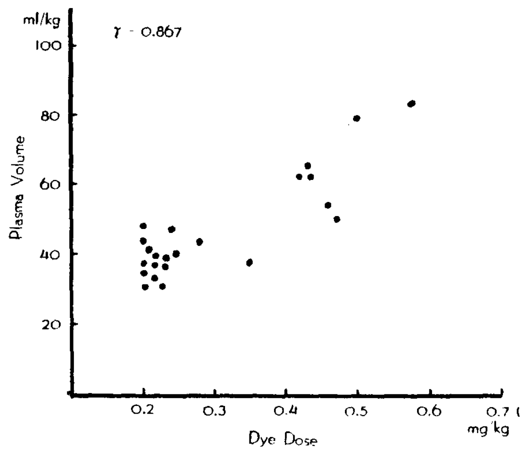


FIG. 10. *Correlation Between Blood Volume Per kg Body Weight Determined by* GIBSON & EVANS' *Method and Injected Dye Dose*

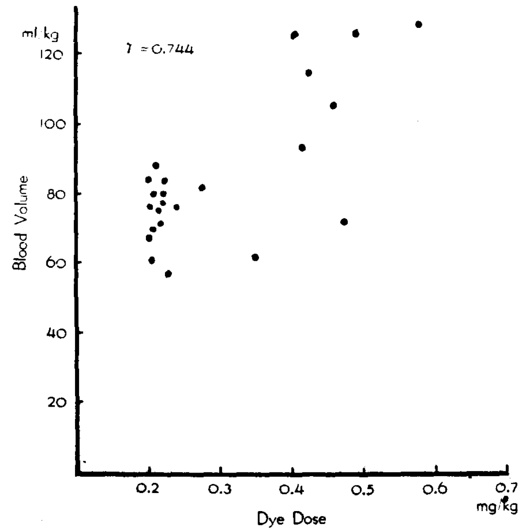


FIG. 11. *Correlation Between Blood Volume Per kg Body Weight Determined by* LAWSON'S *Method and Injected Dye Dose*

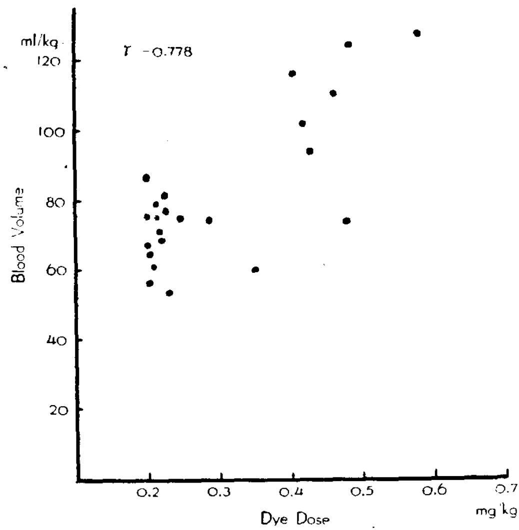


FIG. 12. *Correlation Between Blood Volume Per kg Body Weight Determined by* GREGERSEN'S *Method and Injected Dye Dose*

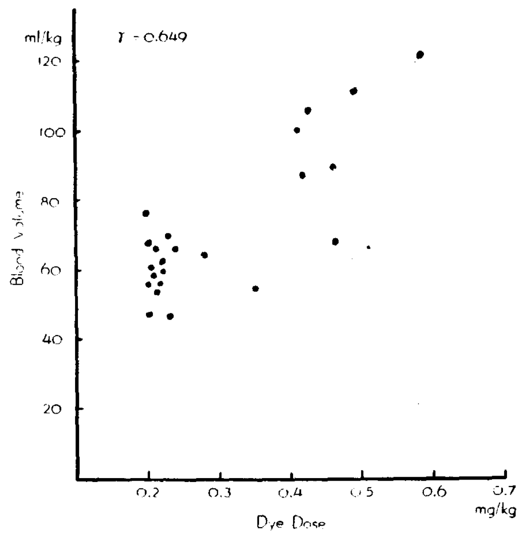


TABLE 9. *Blood and Plasma Volume Determined by LAWSON's Method* (T-1824 0.216~0.248 mg/kg)

HORSE'S NAME	CIRCULATORY BLOOD VOLUME (L)	CIRCULATORY BLOOD VOLUME (ml/kg)	CIRCULATORY PLASMA VOLUME (L)	CIRCULATORY PLASMA VOLUME (ml/kg)
Shirakawa	25.8	55.8	17.0	36.8
Hokuto	27.5	67.0	17.2	41.9
Kooun	33.9	61.6	21.2	38.5
Hatsuhime 1	26.2	48.5	16.9	31.3
Hanahime	30.5	70.9	20.8	48.4
Seijiu	19.1	61.6	12.2	39.3
Riun	42.3	67.1	26.0	41.3
Average	29.3	61.8	18.8	39.6

TABLE 10. *Horses Used in the Experiment and Injected Dose of T-1824* (0.289~0.430 mg/kg)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	Ht (%)
Wakaba	Percheron	♀	9	690	0.289	33.0
Wakaba	"	"	9	700	0.357	30.0
Hatsuhime	"	"	3	595	0.420	42.0
Yamazakura	"	"	8	580	0.430	30.0

TABLE 11. *Blood and Plasma Volume Determined by LAWSON's Method*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME (L)	CIRCULATORY BLOOD VOLUME (ml/kg)	CIRCULATORY PLASMA VOLUME (L)	CIRCULATORY PLASMA VOLUME (ml/kg)
Wakaba	45.4	65.8	31.0	44.9
Wakaba	39.0	55.7	27.8	39.7
Hatsuhime	63.2	106.2	37.8	63.5
Yamazakura	51.4	88.6	36.6	63.1
Average	49.75	79.07	33.30	52.80

TABLE 12. *Horses Used in the Experiment and Injected Dose of T-1824* (0.431~0.586 mg/kg)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	Ht (%)
Yamazakura	Percheron	♀	8	580	0.431	40.0
Hanahime 1	"	"	7	540	0.462	41.0
Kinsei	"	"	3	525	0.475	27.5
Hatsuhime	"	"	3	505	0.495	30.0
Hanahime	"	"	2	426	0.586	32.0

TABLE 13. *Blood and Plasma Volume Determined by LAWSON's Method* (T-1824 0.431~0.586 mg/kg)

HORSE'S NAME	CIRCULATORY BLOOD VOLUME		CIRCULATORY PLASMA VOLUME	
	(L)	(ml/kg)	(L)	(ml/kg)
Yamazakura	62.1	107.0	38.3	66.0
Hanahime 1	48.8	90.4	29.6	54.8
Kinsei	36.3	69.1	26.7	50.9
Hatsuhime	57.5	113.9	40.9	80.9
Hanahime	52.2	122.5	36.2	84.9
Average	51.38	100.58	34.34	67.50

3) Differences coming from sites from which blood samples were taken

In all cases in the present studies, dye was infused through the jugular vein and blood samples were taken from the opposite jugular vein. However, in order to determine the presence or absence of differences according to blood sample sites and also to investigate measurement errors, experiments were made as indicated in table 14; 4 horses were used in the experiment and dye was infused into the jugular vein. Blood samples were taken at regular intervals from the opposite jugular vein and from the forefoot median vein. The extinction was measured and both circulatory blood volume and circulatory plasma volume were obtained by GIBSON & EVANS' method. The volume of dye infused was 0.204~0.495 mg per kg body weight; the course of extinction was charted in fig. 13. As may be seen in that figure, the time lapse of extinction showed no significant difference which could be considered due to site of taking of sample. The courses of circulatory blood volume and circulatory plasma volume obtained by the two blood sample collection methods are shown in table 15. As shown, almost no difference is seen according to the site from which blood samples were collected. Likewise it was shown that no difference resulted from the volume of dye infused. The largest difference seen in circulatory blood volume

due to the two blood sample collection methods was 320 ml. This is probably a result of measurement errors; in the present experiments the error margin is in approximation with that error.

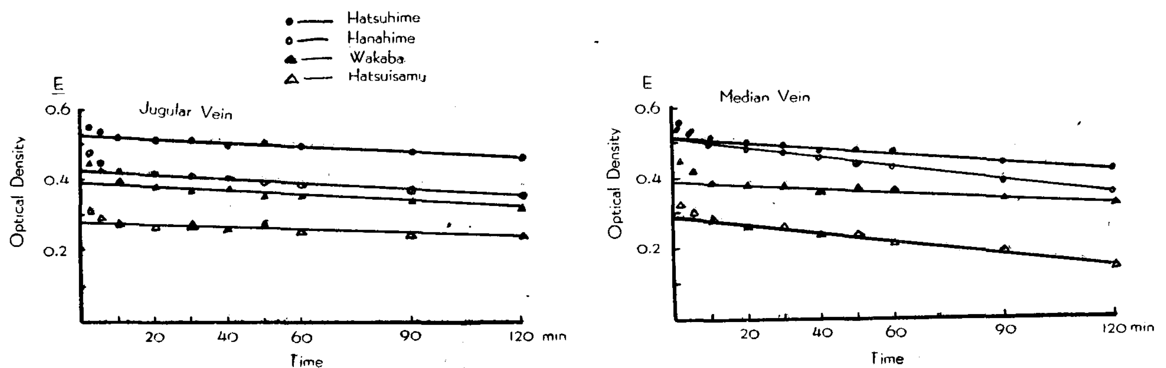
TABLE 14. *Horses Used in the Experiment and Injected Dose of T-1824* (0.204~0.568 mg/kg)

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	AMOUNT OF T-1824 INJ. (mg/kg)	100-Ht ×0.96	Ht (%)
Hatsuisamu	Percheron	♂	9	610	0.204	64.4	37.0
Wakaba	"	♀	9	690	0.289	68.3	33.0
Hanahime	"	"	2	426	0.568	69.2	32.0
Hatsuhime	"	"	3	505	0.495	71.2	30.0
Average				577	0.394	65.7	33.0

TABLE 15. *Blood and Plasma Volume Determined by the Use of Blood from Two Different Positions*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME (L)	CIRCULATORY BLOOD VOLUME (ml/kg)	CIRCULATORY PLASMA VOLUME (L)	CIRCULATORY PLASMA VOLUME (ml/kg)	DRAWN FROM
Hatsuisamu	51.27	84.0	33.25	54.5	V. jugularis
	50.95	83.5	32.10	52.6	V. mediana
Wakaba	55.92	82.5	38.20	55.4	V. jugularis
	55.92	82.5	38.20	55.4	V. mediana
Hanahime	55.23	129.6	38.26	89.8	V. jugularis
	55.19	129.6	37.52	88.0	V. mediana
Hatsuhime	64.37	127.4	45.83	90.8	V. jugularis
	64.37	127.4	45.83	90.8	V. mediana

FIG. 13. *Time Course of Optical Density of Plasma*



4) On the relationship between body weight and circulatory blood and plasma volume

As mentioned hitherto, T-1824 was infused at a rate of 0.202~0.289 mg per kg body weight and the circulatory blood volume and circulatory plasma volume were calculated by the 3 above discussed methods. The results in 5 cases are shown in figs. 14~19 with circulatory blood and plasma volume per kg body weight indicated in the vertical axis and body weight in the horizontal axis. On the supposition that the above distribution is scattered along a straight line, a most appropriate experimental formula may be calculated by least square method. This formula is as may be seen in figs. 14~19. The logarithm of the body weight in the formula indicates the circulatory blood volume and cirulatory plasma volume per kg body weight. This is collectively expressed in table 16. As may be seen all values obtained by the present method, in all individual cases show higher values

TABLE 16. *Blood and Plasma Volume Per kg Body Weight Determined by Least Mean Square*

METHOD	CIRCULATORY BLOOD VOLUME (ml/kg)	CIRCULATORY PLASMA VOLUME (ml/kg)
LAWSON's method	76.0	50.0
GREGERSEN's method	78.0	52.0
GIBSON & EVANS' method	81.0	53.0

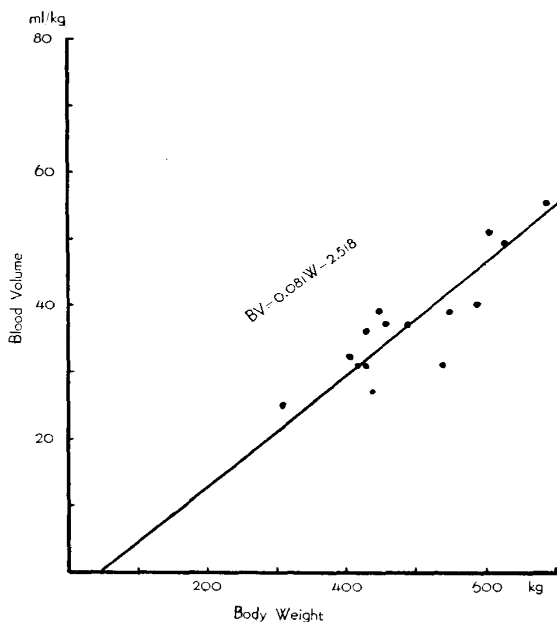


FIG. 14. *Correlation Between Body Weight and Blood Volume Determined by GIBSON & EVANS' Method*

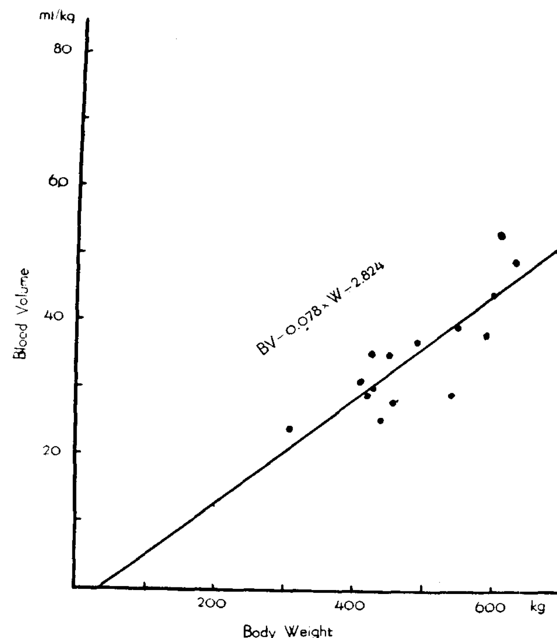


FIG. 15. *Correlation Between Body Weight and Blood Volume Determined by GREGERSEN's Method*

than the mean value of circulatory blood and plasma volume per kg body weight. Thus, it may be said that the values calculated by the present method have a higher degree of accuracy. As shown in table 16 the values LAWSON's method are minimum with 76 ml/kg for circulatory blood volume and 50 ml/kg for circulatory plasma volume.

FIG. 16. *Correlation Between Body Weight and Blood Volume Determined by LAWSON's Method*

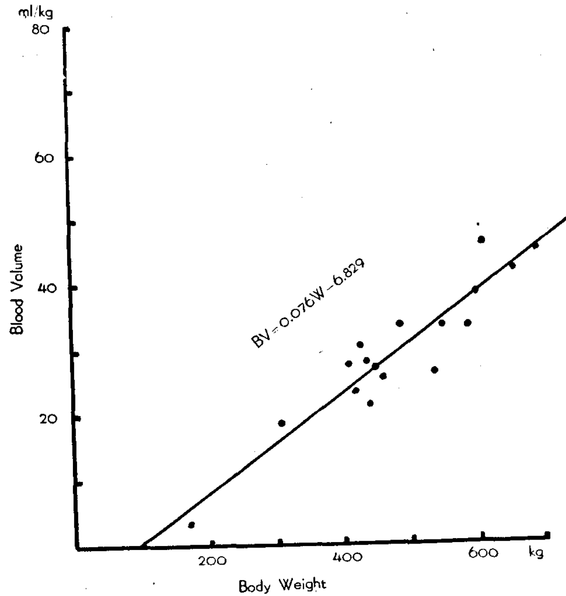


FIG. 17. *Correlation Between Body Weight and Plasma Volume Determined by GIBSON's Method*

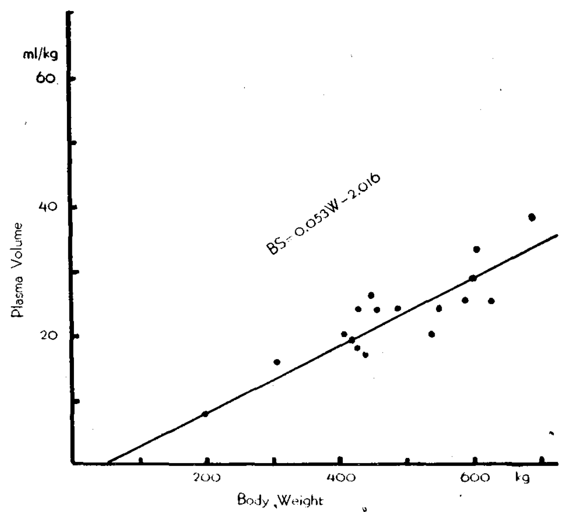


FIG. 18. *Correlation Between Body Weight and Plasma Volume Determined by GREGERSEN's Method*

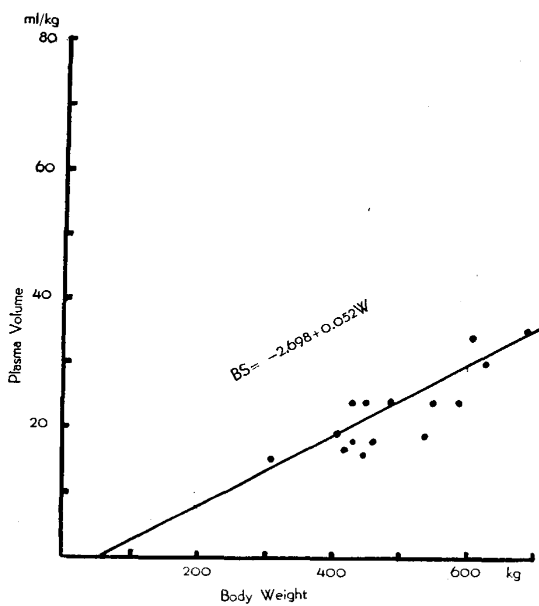
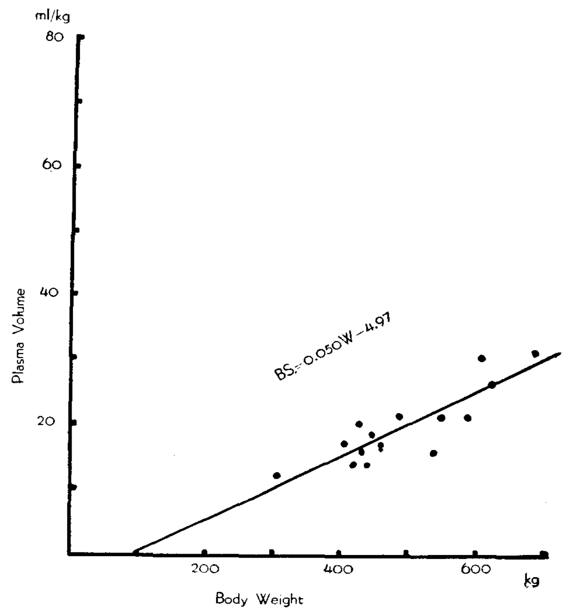


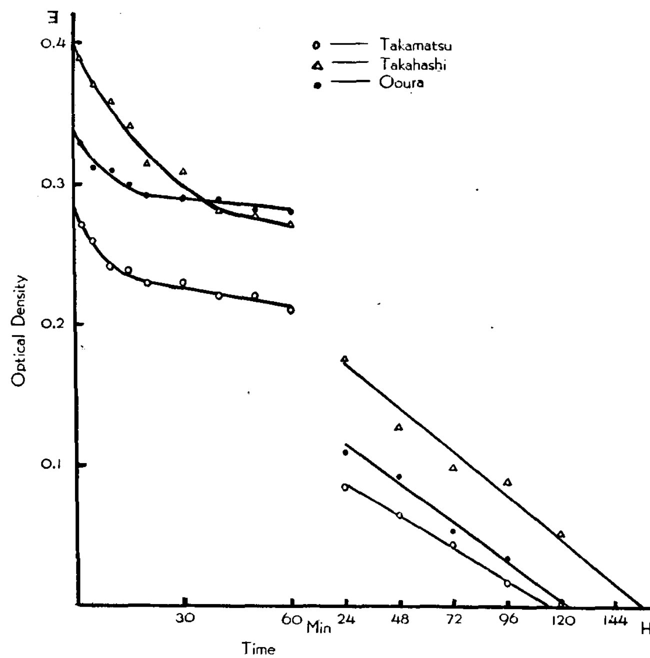
FIG. 19. *Correlation Between Body Weight and Plasma Volume Determined by LAWSON's Method*



5) Disappearance of dye from blood

When the 1 minute value of extinction was calculated by LAWSON's method, the calculation was done by extrapolation to the vertical axis; in opposition to this, when the minute value of extinction is extrapolated to the horizontal axis the time of disappearance may be obtained. Since the experimental formula is obtained by least square method, when the time of disappearance is calculated from this, the shortest time comes to 7 days with the longest at 260 days both of which are extremely high values. Thus in order to investigate the actual course of disappearance of dye from blood, a long-term observation after dye infusion was made on 4 horses. The light absorption quotient was measured in the short period when the circulatory blood volume was measured when blood samples were collected and from 24 hours once every 24 hours up to 120 hours (Fig. 20). However in the figure, since 2 cases became superimposed and therefore hard to tell apart, only 3 out of the 4 cases are charted. As may be seen in the figure within a short period, as well known, there is a period when the extinction decreases exponential functionally and a period when the extinction decreases in a straight line which after 24 hours continues to decrease in a straight line and disappears at 5~6 days.

FIG. 20. *Time Course of Optical Density*



When the course of disappearance depicted as a straight line is extrapolated to zero minutes, in all cases, the values are smaller than those of the extinction after 60 minutes.

Based on the above, it may be said that the decreasing of extinction has 3 phases. 1) A rapid exponential function decrease period. 2) A rapid decreasing period when the dye adheres to the blood vessel walls and when disintegration occurs. 3) A gradual decreasing period when a comparatively slow straight line decrease is seen after 24 hours.

TABLE 17. *Change in Ht Value*

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	TIME (min)											
					5	10	15	20	30	40	50	60	90	Average	Maxi-mum	Mini-mum
Hatsuisamu	Pcreheron	♂	9	610	37.40	37.20	37.00	36.80	37.60	38.00	38.00	38.00	38.20	37.57	38.20	36.80
Wakaba	"	♀	9	690	33.40	33.00	32.40	32.80	33.40	33.00	33.00	33.00	33.20	33.02	33.40	32.40
Hanahime	"	"	7	540	37.00	36.80	36.80	37.20	37.20	37.20	37.40	38.00	38.20	37.31	38.20	36.80
Hatsuhime	"	"	3	490	36.20	36.00	35.80	35.60	36.00	36.00	36.40	36.40	37.40	36.20	37.40	35.60
Elizabeth	Norman	"	4	445	36.60	36.20	36.20	35.80	36.00	36.00	36.60	36.80	37.40	36.40	37.40	35.80
Shirakawa	"	"	14	462	37.00	36.80	37.00	36.40	37.20	37.60	37.60	38.00	38.00	37.28	38.00	36.40
Hokuto	"	♂	14	410	40.00	39.40	39.40	39.00	39.00	39.00	39.20	39.60	40.00	39.40	40.00	39.00
Appudēru	"	"	10	438	42.00	23.60	23.60	23.00	23.20	23.20	24.00	23.20	24.20	25.55	42.00	23.20
Tokushima	"	"	10	462	32.40	42.00	41.80	41.60	42.20	43.00	42.80	42.80	43.20	41.31	43.20	32.40
Yoshitaka	"	"	12	450	30.60	30.40	30.00	30.20	30.40	29.80	30.40	30.60	31.00	30.37	31.00	29.80
Hokuto	"	♂	14	440	36.40	39.80	41.00	41.00	41.00	41.20	41.80	41.80	42.40	40.71	42.40	36.40
Average				494	36.27	35.56	35.55	35.40	35.75	35.82	36.11	36.20	36.66	35.92	38.30	34.05

6) Fluctuations of haematocrit (Ht) values

Since Ht values are used when circulatory blood volume is calculated, the values are influenced by those Ht values. Also since some of the horses raise a commotion during blood samples taking, there is a strong possibility that Ht values change in such a case. Therefore, part of the blood samples taken at the time of circulatory blood volume measurements from 11 horses was measured for Ht values and the fluctuations in values and measurement errors were investigated. The results are shown in table 17. The fluctuations as shown, are assumed to include measurement errors and physiological changes; it was noted that considerable differences were present. Thus, such fluctuations in Ht values may enter into the measurements of circulatory blood volume and cause a considerable marginal error.

7) Measurement of circulatory blood volume and circulatory plasma volume by ^{32}P

As shown in table 18, 6 horses were used in the ^{32}P measurements of circulatory blood and plasma volume. Out of the 6 cases 3 were investigated simultaneously with T-1824.

TABLE 18. *Horses Used in the ^{32}P Experiment*

HORSE'S NAME	BREED	SEX	AGE (Year)	BODY WEIGHT (kg)	Ht (%)
Kihisa	Percheron	♀	8	600	38.9
Isamu	"	"	16	462	37.8
Riun	"	"	12	540	40.2
Elizabeth	Norman	"	4	445	36.0
Appuderu	"	♂	10	438	41.0
Yoshitaka	"	"	12	450	34.0

TABLE 19. *Blood Volume Determined by ^{32}P Method* (whole blood method)

HORSE'S NAME	CIRCULATORY BLOOD VOLUME		RCV		CIRCULATORY PLASMA VOLUME	
	(L)	(ml/kg)	(L)	(ml/kg)	(L)	(ml/kg)
Kihisa	43.1	71.9	16.3	27.2	26.8	44.68
Isamu	35.4	76.5	12.9	28.1	22.4	48.48
Riun	34.3	63.5	13.4	24.8	20.9	38.70
Elizabeth	35.3	79.3	12.3	27.6	22.9	51.66
Appuderu	32.1	72.3	12.8	29.2	19.3	44.06
Yoshitaka	34.9	77.9	11.5	25.5	23.4	52.00
Average	35.85	73.56	13.20	27.06	22.62	46.95

RCV: Red cell volume

TABLE 20. *Blood and Plasma Volumes Determined Simultaneously by Dye and ^{32}P Method*

	HORSE'S NAME	LAWSON'S METHOD		GREGERSEN'S METHOD		GIBSON & EVANS' METHOD		^{32}P METHOD	
		(L)	(ml/kg)	(L)	(ml/kg)	(L)	(ml/kg)	(L)	(ml/kg)
Circulatory blood volume	**Elizabeth**	31.2	70.1	36.0	81.0	37.5	84.4	35.3	79.3
	Appuderu	30.0	68.5	35.6	79.9	33.9	77.6	32.1	73.3
	Yoshitaka	23.7	79.5	35.7	79.5	39.9	88.7	34.9	77.6
	Average	29.5	72.7	35.6	80.1	37.1	83.5	34.1	76.7
Circulatory plasma volume	**Elizabeth**	20.4	45.9	23.6	53.2	24.4	54.9	22.9	51.6
	Appuderu	18.2	41.7	21.2	48.5	20.4	46.7	19.3	44.1
	Yoshitaka	18.3	40.9	24.1	53.6	26.8	59.8	23.4	52.0
	Average	19.0	42.8	23.0	51.7	23.9	53.8	21.8	49.2

The results of the ^{32}P measurements in the 6 cases are shown in table 19. As may be seen the mean value of circulatory blood volume per kg body weight was 73.56 ml, the same in blood corpuscle volume was 27.06 ml and the same in circulatory plasma volume was 46.95 ml. When these values were compared with those of the 3 cases in which the dye method was used, the results were as seen in table 20. It is noted that in both circulatory blood volume and circulatory plasma volume use of GREGERSEN's and GIBSON & EVANS' methods produced higher values as compared with values obtained by the ^{32}P method while those obtained by LAWSON's method were smaller. When the ratio of the values obtained by 3 dye methods to ^{32}P total blood method was calculated the results were as shown in table 21. Further, when ^{32}P total blood method was compared with blood corpuscle method the results were as seen in tables 22~24. The values in blood corpuscle method are slightly higher than those obtained by the total blood method.

TABLE 21. *Comparison of Values Determined by Dye and ^{32}P Methods* (whole blood method)

HORSE'S NAME	$\frac{^{32}P - \text{LAWSON METHOD}}{^{32}P} \times 100$		$\frac{\text{GREGERSEN} - ^{32}P}{^{32}P} \times 100$		$\frac{\text{GIBSON \& EVANS} - ^{32}P}{^{32}P} \times 100$	
	Circulatory blood volume	Circulatory plasma volume	Circulatory blood volume	Circulatory plasma volume	Circulatory blood volume	Circulatory plasma volume
Elizabeth	11.4	11.3	1.9	3.0	6.5	6.0
Appuderu	6.8	5.1	2.7	9.9	5.9	6.2
Yoshitaka	21.7	2.1	2.5	2.9	1.4	10.6
Average	13.3	6.2	2.4	5.3	4.6	7.6

TABLE 22. *Comparison of Values Determined by Dye and Two ^{32}P Methods* (whole blood and red corpuscle methods)

HORSE'S NAME	RCV + SV ^{32}P T-1824	$\frac{\text{CBV } ^{32}P}{\text{CBV T-1824}}$	$\frac{\text{CBV } ^{32}P}{\text{TRUE CBV}}$	$\frac{\text{CBV T-1824}}{\text{TRUE CBV}}$
Elizabeth	37.138	0.940	0.951	1.006
Appuderu	31.848	0.944	1.007	1.066
Yoshitaka	37.216	0.888	0.938	1.073
Average	35.400	0.924	0.965	1.048

CBV: Circulatory blood volume
RCV: Circulatory red cell volume
SV : Serum volume

TABLE 23. *Comparison of Blood and Plasma Volume Per kg Body Weight Obtained by the Three Methods*

HORSE'S NAME	CIRCULATORY BLOOD VOLUME		CIRCULATORY PLASMA VOLUME	
	GIBSON & EVANS–LAWSON	GIBSON & EVANS	GIBSON & EVANS–LAWSON	GIBSON & EVANS
Hatsuisamu	4.7 L	8.9 %	3.3 L	6.4 %
Wakaba	10.5	18.8	7.2	12.8
Hatsuhime 1	5.6	17.6	3.6	11.3
Hatsuhime	4.4	11.6	2.9	7.7
Yamazakura	7.4	18.1	4.7	11.5
Hanahime	5.9	16.2	4.1	11.2
Elizabeth	5.9	21.5	3.8	13.9
Shirakawa	11.2	30.2	7.2	19.9
Hokuto	4.6	14.3	2.8	8.7
Appuderu	4.0	12.8	2.3	7.4
Tokushima	8.0	25.1	4.8	15.0
Yoshitaka	12.6	31.6	8.5	21.3
Seijiu	5.9	13.7	3.8	15.2
Kooun	5.6	14.1	3.5	8.8
Riun	6.8	13.8	4.2	8.5
Average		17.9		11.9

TABLE 24. *Blood Volume Determined by ^{32}P Method*

HORSE'S NAME	CIRCULATORY BLOOD CELL VOLUME / CIRCULATORY BLOOD VOLUME	Ht	CIRCULATORY BLOOD CELL VOLUME / Ht
Kihisa	37.8 %	38.9 %	0.972 %
Isamu	36.6	37.8	0.970
Riun	39.1	40.2	0.973
Elizabeth	34.8	36.0	0.967
Appuderu	39.9	41.0	0.974
Yoshitaka	33.0	34.0	0.971
Average	36.9	37.9	0.971

DISCUSSION

When circulatory blood volume is measured by the dye method, it becomes necessary to determine blood dye concentration, prior to the disappearance of infused dye from blood and at the time when dye and blood are mixed evenly.

However, since in actuality it is impossible to collect serum at this point, methods by which blood dye concentration of a corresponding period can be obtained theoretically, have been developed. Thus, GIBSON & EVANS observed the time course of blood dye concentration following infusion of T-1824 and by extrapolating to 0 minute from the disappearance curve which comes after the mixing curve, they assumed that it was possible to calculate a theoretically accurate dye concentration of the blood and dye mixing period. In addition GREGERSEN advocated a clinical simplified 10 minute method based on the fact that at 10 minutes after dye infusion the blood dye concentration generally coincides with the GIBSON & EVANS extrapolation. On the other hand, as compared with the radioisotope method, it is known that use of the EVANS blue method and GREGERSEN's method resulted in higher values. Further, LAWSON et al. using dogs observed the shift of blood dye concentration following dye infusion. Utilizing the exponential slope which followed in wake of the rapid disappearance phase, LAWSON set forth his so-called dye-decrement method and reported that the plasma volume calculated by this method showed values approximately 15% lower than that obtained by the GIBSON & EVANS' method. According to LAWSON this is due to the fact that immediately after infusion, EVANS blue adheres to blood vessel walls and thus decreases the blood dye concentration, as a result of which plasma volume is calculated in excess. In addition, LAWSON et al.[16] discovered that in the rapid disappearance phase the logarithm of the dye concentration was in a linear relation with the logarithm of time and set forth a method by which minute values of dye concentration could be extrapolated. LAWSON et al. reported that the values obtained by this method coincided with those by the dye-decrement method. When the above methods set forth by workers hitherto are observed from a theoretical view point, it is apparent that in the GIBSON & EVANS' method it is assumed that the change $C(t)$ in blood dye concentration is in a linear relation with time t, namely it is assumed that $C(t)=at+b$. In this expression a and b are constant. In contrast to this, in LAWSON's method in his rapid disappearance phase, it is apparent that LAWSON assumed the relationship $\log C(t)=e\log(t)+f$.

Both e and f are constant. Therefore it follows that in LAWSON's method the relationship $C(t)=a't^n+b'$ is assumed. In this expression a', b', n are respectively constant. Now in GIBSON & EVANS' method since $C(0)=b$ of zero minute values are calculated, it is theoretically possible to understand. However, in LAWSON's method in spite of the fact that $C(1)=a'+b'$ of 1 minute values are being calculated, since theoretically there is no reason for taking 1 minute values, it may be said that LAWSON's method is based on experience.

Now if GREGERSEN's method is assumed to be correct, $C(10)=10a+b$ and the difference between results derived from GIBSON & EVANS' method would be $10a$.

Therefore, this would mean that the difference between GIBSON & EVANS' method would be influenced by coefficient *a*, namely by the direction cosine. In the present experiment 15 healthy horses were used. When the dye infusion volume was 0.202~0.289 mg per kg body weight the mean values of circulatory blood volume per kg body weight were found by LAWSON's method to be 62.0 ml, by GREGERSEN's method 72.2 ml and by GIBSON & EVANS' method 76.0 ml. At the same time, the mean values of circulatory plasma volume per kg body weight were by LAWSON's method 40 ml, GREGERSEN's method 46.4 ml and by GIBSON & EVANS' method 49.0 ml.

In other words, the order of the values in circulatory blood and plasma volume were as follows: Those obtained by LAWSON's method, GREGERSEN's method, GIBSON & EVANS' method. In circulatory blood volume GREGERSEN's method showed an average of 16.9% higher values as compared with LAWSON's method; by GIBSON & EVANS' method 23.6% higher values were obtained. In circulatory plasma volume GREGERSEN's method showed an average of 16.9% higher values as compared with those gained by LAWSON's method, and by GIBSON & EVANS' method 23.6% higher values were obtained. The circulatory blood and plasma volumes determined by LAWSON's method were 17.9% and 11.9% lower than those by GIBSON & EVANS' method respectively. However in experiments using dogs, LAWSON et al., reported that the circulatory plasma volume gained by LAWSON's method was 15% lower than that by GIBSON & EVANS' method; in horses the reduction rate was smaller than that. This is probably due to the difference in animals; especially, in the rapid disappearance slope the horse shows a longer period of time as compared with the dog and therefore, a difference in extrapolatory point occurs which accounts for the difference in obtained value of reduction rate.

When ^{32}P total blood method which is reported to give comparatively accurate values, is compared with dye method, both GREGERSEN's and GIBSON & EVANS' method show higher values than obtained by the ^{32}P method; by LAWSON's method which he believed to be comparatively accurate, lower values as compared with those by ^{32}P were obtained. In the present experiment, as may be seen in table 19 GREGERSEN's method yielded the values closest to those obtained by ^{32}P method. From experiments on circulatory blood and plasma volume by the use of various volumes of infused dye, in the present work it must be noted that a proportional increase in circulatory blood volume to the volume of infused dye is seen. This indicates that the dye is taken up by the blood vessel walls and other sites. In spite of the fact that within the range of 0.200~0.250 mg per kg body weight infused dye volume, the above tendency is comparatively hard to find; to a certain extent the above phenomenon is universal. Thus the present author is of the opinion that in the dye method when 0 minute values are to be extrapolated by

theoretical means, correction values making allowance for the inclination of the decrement slope and blood wall dimensions should be calculated by some means or other. Although, as to how this correction may be made, there is no answer yet, the author wishes to point out strongly in regard to the dye method, the necessity of setting forth correction values with the above mentioned points taken into consideration and dye specific or animal specific adaptations made. With regard to the results of long term observations on disappearance of dye, it was found that the infused dye after passing through the mixing phase, for a certain period showed a rapid straight line decrease. In findings reported in the present paper, studies were made for only 2 hours, but after 24 hours a gradual straight line decrease was indicated. The difference in disappearance rate as described above, is conjectured to be brought about by adherence to blood vessel walls and by concentration of the dye which remains in the blood. Therefore, in the measurement of circulatory blood by the dye method, in order to obtain accurate values, the volume of infused dye must be taken into consideration. Likewise in the calculation of circulatory blood volume from circulatory plasma volume, Ht values are used, therefore accurate Ht values must first be obtained. However, it is known that Ht values in animals show remarkable changes caused by excitement, tension, etc.[2,22]

In the present experiments, as a result of measuring Ht values of part of the blood samples taken at given intervals for the purpose of measuring blood dye volume, some samples showed remarkable changes, which caused the author to doubt the advisability of using the said Ht values. GREGERSEN reported that the centrifuged blood corpuscle volume still contained 4% of plasma and that the correction values should be 0.96; he found that there was an 8～9% error in CHAPIN & EVANS' centrifuged Ht value. On the other hand, there is a report which states that venous blood Ht values do not necessarily express the ratio of blood corpuscles and plasma. In the author's ^{32}P experiments, as to the percentage corresponding to Ht values from circulatory blood and plasma volume in total blood method, the average was 36.9%. Further the author indicated that the correction value was 0.971 which is slightly higher than GREGERSEN's correction value 0.96% and identical to JENNINGS' correction value. It may be said that in horses, JENNINGS' correction value[14] is appropriate. In measurement experiments with ^{32}P, total blood method and blood corpuscle method were studied. As a result in blood corpuscle method, circulatory blood corpuscle volume is calculated to which circulatory plasma volume obtained by dye method is added. Consequently not only it is impossible to avoid shortcomings in the application of the dye method but also, because of the complicated procedure, experimental errors may be expected to be comparatively larger. In the present experiments the values

derived from the blood corpuscle method were higher than the values obtained by total blood method, this may also be due to similar reasons. In the present experiments, among the measurements for circulatory blood volume the most accurate values are obtained by the total blood method, that is to say the values obtained by ^{32}P labeled corpuscle method in total blood are the most reliable.

SUMMARY

Studies on methods of measurement of circulatory blood volume and circulatory plasma volume of horses by the dye method and the ^{32}P method were made on 32 healthy horses; the following summarized results were obtained.

1) When values obtained by LAWSON, GREGERSEN, and GIBSON & EVANS using T-1824 were compared, within a range of 0.202~0.289 mg per kg body weight GIBSON & EVANS' method showed the highest values in both circulatory blood volume and circulatory plasma volume. Circulatory blood volume per kg body weight obtained by LAWSON's method was 62.0 ml, by GREGERSEN's 72.2 ml, by GIBSON & EVANS' 76.0 ml. Circulatory plasma volume by LAWSON's was 40.0 ml, by GREGERSEN's 46.4 ml, by GIBSON & EVANS' method 49.0 ml.

2) When circulatory blood volume and circulatory plasma volume changes corresponding to dye volume changes within a range of 0.202~0.586 mg were studied, within the range 0.202~0.250 no significant changes were seen. However when the volume of infused dye was increased, the values of circulatory blood volume and plasma volume increased with the increase of volume of infused dye.

3) Dye was infused through jugular vein and blood samples were taken at regular intervals from the opposite jugular vein and right forefoot median vein; the differences by sampling site were studied. It was found in regard to the time course of extinction and the circulatory blood volume and plasma volume obtained from the time course of extinction, that no significant differences were seen by sampling site.

4) From the correlation between circulatory blood volume and circulatory plasma volume and body weight obtained from infused dye volume within a range of 0.202~0.289 mg per kg body weight by least square method, circulatory blood volume and circulatory plasma volume per kg body weight calculated.

5) One minute values of extinction were calculated by LAWSON's method, by extrapolation to the vertical axis. When extrapolated to the horizontal axis the time of disappearance was obtained. Since an experimental formula can be expressed by least square method when the time of disappearance is calculated by this formula, the time required at its shortest was 7 days and the same at its longest was 260 days. However, in actuality when long term observations were made on plasma dye concentration it was found that the time of disappear-

ance was 5~6 days.

6) When circulatory blood volume was measured, samples were taken at regular intervals; Ht values were measured from part of the samples and their changes were studied. It was noted that besides experimental error, considerable physiological changes occurred.

7) In the measurement of circulatory blood volume by ^{32}P especially in blood corpuscle method the total blood volume value was 73.56 ml per kg body weight while by use of total blood method the obtained value was 46.95 ml; thus the blood corpuscle method showed higher values than total blood method.

When the ^{32}P method and the dye method were compared, it was noted that the values by GREGERSEN's method were closely approximate to those obtained by the ^{32}P method. When compared with simultaneous measurement by T-1824, it was shown that in both circulatory blood volume and circulatory plasma volume, GREGERSEN's and GIBSON & EVANS' methods showed higher values than those obtained by ^{32}P method while by LAWSON's method the values were smaller.

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ANNOUNCEMENT

In 1960, from August 28 to September 3, the **First International Congress of Histochemistry and Cytochemistry** will be held in **Paris.** It is organized under the auspices of the《Société Française d'Histochimie》in collaboration with the histochemical societies in existence all over the world, especially the《American Histochemical Society》, the《Deutsche Arbeitsgemeinschaft für Histochemie》, the《Société Belge d'Histochimie》, the Italian and Japanese histochemical societies and several non-autonomous sections of histochemistry.

The provisional program will be as follows:

1. **PHYSICAL PROBLEMS** (Chairman: VOSS, Germany)

Topic	Speaker
Ultraviolet spectography	SANDRITTER, Germany
Infrared spectography	LECOMTE, France
Visible spectography and photometry	LOCQUIN, France
Radioautography as a histochemical tool	LEBLOND, Canada
Mass spectography and spectography by X-rays Interferométrie	RINGERTZ, Sweden
Fluoroscopy	DE LERMA, Italy
Electron microscopy	BARRNETT, USA

2. **BIOCHEMICAL DATA APPLIED TO HISTOCHEMISTRY** (Chairman: LILLIE, USA)

Topic	Speaker
Proteins	LISON, Brazil
Phosphatases (specific and non specific)	BANKOWSKI & VORBRODT, Poland
Hydrogen carriers	NOVIKOFF, USA
Esterases	BURSTONE, USA
Derivatives of oxidation and lipides	POLONOVSKI, France, & WOLMAN, Israel
Polysaccharides	TAKEUCHI, Japan
Conjugated proteins and nucleic acids	BRACHET, Belgium
Inorganic histochemistry	HINTZSCHE, Switzerland
Phenols and indols	VIALLI, Italy
Iron Metabolism	GEDICK, Germany
Immuno-histochemistry	MAYERSBACH, Austria

3. **APPLIED HISTOCHEMISTRY** (Chairman: SEKI, Japan)

Topic	Speaker
Connective tissue	DELAUNAY, France—ASBOE-HANSEN, Denmark & SZIRMAI, Holland
Embryonic development	ROSSI, Italy
Metamorphoses and regeneration	
Malignant tumors	GODLEWSKI, Poland & VENDRELY, France
Genital apparatus and placenta	PANIGEL, France
Tooth	WEILL, France
Endocrine glands	HERLANT, Belgium
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