FLUORESCEIN CIRCULATION TIME AS A PROGNOSTIC SIGN IN EXPERIMENTAL TRAUMATIC SHOCK*‡

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Those who have studied traumatic shock have made repeated attempts to find some reliable early sign which would indicate whether a particular animal would, if untreated, die or survive. Such attempts have embraced most of the evident signs of shock. Various investigators have attempted to rely on blood pressure, on the heart rate, on changes in hematocrit, on the state of sensorium, on body temperature, or upon combinations of these signs. None of these has proved to be reliable.

Since the primary features of the syndrome of experimental traumatic shock are those of a progressive failure of the circulatory system, we have sought a prognostic index in a test which includes a number of the functional aspects of this system. We have found that changes in circulation time, as determined by the fluorescein method within the first 2 hours following the traumatic procedure, is a simple yet sensitive early sign in the pattern of slowly developing shock.

Methods

Normal mongrel dogs weighing 6 to 15 kilos were used. On the day before each trauma experiment, control plasma volume, hematocrit readings, and circulation time determinations were made. Thereafter, water was given *ad libitum*, but food was withheld.

The plasma volume was determined by the dye (T-1824) dilution method (Gregersen and Stewart, 1939) with extrapolation of the time-concentration curve on a semilogarithmic plot (Gregersen and Rawson, 1943). Hematocrit values were obtained after centrifuging heparinized blood in Wintrobe tubes for 30 minutes at 3000 R.P.M. The total blood volume was calculated from the measured plasma volume and the hematocrit value (Root, Roughton, and Gregersen, 1945, 1946). The circulation time was determined by rapidly injecting through a 20 gauge needle 1 cc. of a 10 per cent fluorescein solution into the femoral vein of the animal. The time taken for the fluorescent color to reach the conjunctival mucosa, observed in a dark room under an ultraviolet light beam, was recorded as the circulation time (Fishback, 1941). In the normal dog, the fluorescence appeared first in the vicinity of the larger vessels of the conjunctival mucosa and within a fraction of a second the entire conjunctiva, particularly around the edges, suddenly acquired a greenish yellow hue. The appearance of

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the latter was considered as the end-point of our determinations. Within 30 minutes, the fluorescence had diminished sufficiently in the normal animal to permit a second determination with a clear cut result. In a few animals the cyanide circulation time was also determined by using 1 cc. of a 0.1 per cent solution of sodium cyanide (Robb and Weiss, 1933).

On the day of the experiment the animal was placed on its back in a cradle. The control heart rate and rectal temperature were recorded. Mean blood pressure was determined by needle puncture of the femoral artery. The animal was then given ether for 10 to 30 minutes, during which time it was removed from the cradle and both thighs contused with a rawhide mallet weighing about 200 gm. (Gregersen and Root, 1942). After trauma the animal was put back in the cradle and ether was discontinued. The femoral artery and vein were then exposed under local anesthesia (2 per cent procaine). The plasma volume was again determined about 1 hour after injury. The dye was injected into the exposed femoral vein and subsequent blood samples were withdrawn from the femoral artery. Thereafter, determinations of the mean blood pressure and heart rate were made every 20 minutes, and those of circulation time, hematocrit, and rectal temperature were made at intervals of 1 hour. The determinations were continued until death occurred or for a period of 6 hours. If the animal died within 6 hours, a gross autopsy was performed. When the animal survived beyond the 6th hour, the wound in the femoral region was closed, the dog was returned to a cage, and given water. Animals which were alive 24 hours later were counted as survivals.

In ten of the experiments, 1 cc. of blood was withdrawn in heparinized syringes from both the femoral artery and the jugular vein at an hourly interval. The oxygen content of this blood was determined by a microgasometric method (Roughton and Scholander, 1943).

RESULTS

In forty-one normal dogs, the circulation time as determined by the fluorescein method ranged from 9 to 16 seconds with an average of 12.6 seconds and a standard error of 0.3 second. Of these animals, twenty-nine were confused and the circulation times measured before and after injury are included in Table I. After muscle trauma the end-point was not as sharp as in normal animals. It sometimes took 1 second or longer for the maximal hue to develop. In animals in deep shock the error of the end-point may have amounted to as much as 2 seconds. In our experience the circulation time determined immediately following ether and muscle trauma was usually greater than 40 seconds; this has no prognostic significance. As a routine procedure, therefore, the first circulation time measurement was carried out at least one-half hour and usually 1 hour following trauma and was repeated at approximately 1 hour intervals. It was difficult, in the shocked animal, to repeat the procedure oftener since the conjunctiva remained at full hue for some time. This limitation, however, was seldom of consequence for the animals did not usually show much change in less than 1 hour.

The results obtained on a series of thirty-one dogs (Table I) show that in many cases a true prognosis can be made on the basis of two consecutive determinations of the circulation time. In general, it can be said that animals with impending shock in which the circulation time shows a gradual prolongation will die, whereas animals that survive show a slow improvement in the circulation time (see Discussion). An excellent example is illustrated in the

TABLE I

Repeated Determinations of Fluorescein Circulation Time in Traumatized Dogs

Dog No. and sex	Body weight	Blood volume		Fluorescein time in seconds							Duration of survival and other remarks
		Residual volume	Reduction	Control	No. of hrs. after trauma						
					0–1	1–2	2–3	3–4	4–5	5–6	
Deaths											
	kg.	*cc./kg.*	*per cent*								*hrs.*
1♀	14.9	55	29	12		25	43	45	55	75	6
2♀	11.6	75	29	15		37	37	40	53	71	6
3♀	7.1	61	21	12	30	32	37	42	92		4.5
4♀	6.4	67	42	13	23	30	30	70			3.9
5♀	9.3	68	33	15		42	52	98			3.7
6♀	10.0	78	33	14		36	37	50	53	80	6
7♀	7.0	56	32	13	49	56	67				3.3
8♂	8.1	63	38	12		42					2.2
9♂	9.6	68	27	13		23		32	42	60	6
10♀	14.6	70	32	16		35	46				3.6
11♀	11.6	56	29	13			33		33	200?	5.5
12♀	8.3	64	40	9		44					2.8
13♂	8.8	65	25	11		45					2.2
14♂	11.4	63	29			32	28	95			3.7 See discussion
15♀	6.7	90	12	13		34	64				2.7 Femur fractured
16♂	10.9			11		28		36	56		5.2
17♂	9.3	70	31	9		21		34			6
18♂	10.1	56	34	15			42		46		6
19♀	8.5	68	36	10		33					2
20♀	10.0	60		10		29	39				2.3
Survivals											
21♀	7.1	92	16	16		24				29	
22♀	8.1	87	31	13		18	21	20 (more blows)			
									49	31	
23♂	9.9	70	32	11		29	27		19		
24♀	11.1	79	30	11		22		16		16	
25♂	10.9	65	34			29		26	27		
26♀	8.3	75	29	14		38	38		38	38	See discussion
27♂	9.0	80	29	14		30	26	25		21	
28♂	11.1	65	29	13		19	24	40		52	See discussion
29♂	9.3	65	35	16		29		30	28	28	
30♂	13.9	72	23	13		53	38	35		34	
31♀	12.6	70	30	9		29		18	21	19	

charts of dogs 3 and 27 (Fig. 1). The heart rate and mean blood pressure of the two animals are comparable for the first 4 hours. During this period, however, the fluorescein circulation time in dog 3 shows a gradual prolongation, while

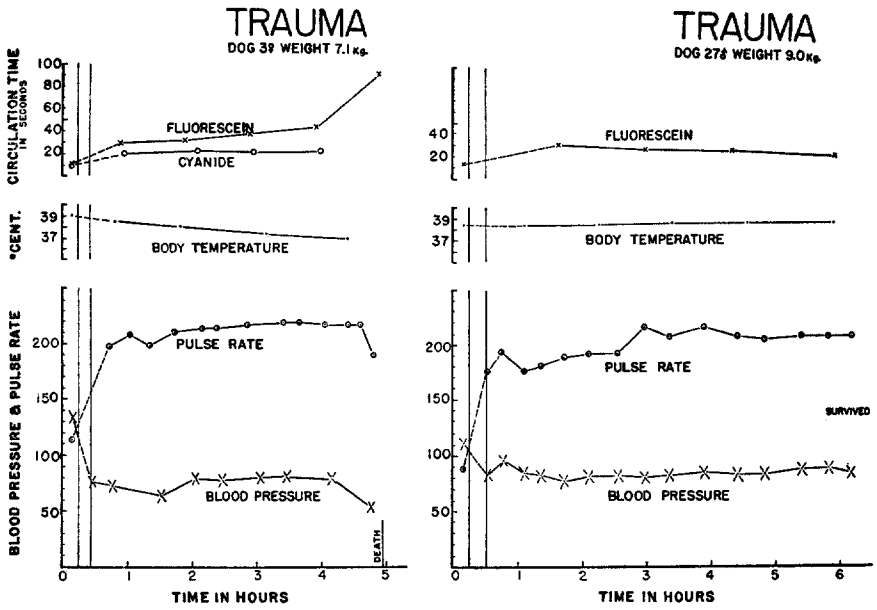

FIG. 1. Comparison of the body temperature, pulse rate, and mean blood pressure with the fluorescein circulation time in dog 3 that died and dog 27 which survived. Repeated determinations of cyanide circulation time were also made on dog 3. No cyanide response could be obtained just before death. See also Table II.

TABLE II

Comparison of Fluorescein (F) and Cyanide (CN) Circulation Times in Traumatized Dogs

Dog No. and sex	Circulation times in seconds							Duration of survival and other remarks
	Control	No. of hrs. after trauma						
		0–1	1–2	2–3	3–4	4–5	5–6	
2 ♀	(F) 15		37	37	40	53	71	Died shortly after last (F) determination
	(CN) 14		22	20	24	27	23	
3 ♀	(F) 12	30	32	37	42	92		Died during last (CN) determination
	(CN) 11	20	23	21	23	No effect		
4 ♀	(F) 13	23	30	30	70			Died shortly after last (CN) determination
	(CN) 11	20	20	24	24, 35			

that of dog 27 shows a gradual return to the control value. The former sustained a sudden drop in blood pressure and died in the 5th hour whereas the latter survived. In our experience, the change in rectal temperature is of no great

importance, for it depends among other things upon the room temperature (see also Gregersen and Root, 1946).

In comparing the cyanide circulation time with the fluorescein time we found that the cyanide circulation time, although increased in shock, remained fairly constant (around 25 seconds), whereas the fluorescein circulation time showed progressive changes over the same period in the same traumatized animal (Table II and Fig. 1, dog 3).

In ten of the dogs the oxygen contents of the femoral arterial and the jugular venous blood were determined (Table III). In general, there is a fair correlation between the change in the A-V oxygen difference and that in the fluorescein circulation time. However, in several experiments the A-V oxygen difference in the 1st hour following trauma was small, and did not reflect accurately the severity of injury (see Discussion).

DISCUSSION

That an animal after receiving moderate to severe muscle trauma will show an impaired circulation (Cannon, 1923) and consequently a prolonged circulation time is well known (Olson, Gutmann, Levinson, and Necheles, 1941). In our experience, the prolonged cyanide circulation time is maintained with fair constancy until late in the course of shock when the mean blood pressure falls below 50 mm. Hg (Table II). This confirms the previous finding of Olson *et al.*, that in their series of twenty-three experiments most cyanide circulation time readings after muscle trauma were less than 30 seconds. Of the few animals reported by them as having a circulation time longer than 30 seconds, nearly all had mean blood pressures below 50 mm. Hg. On the other hand, the fluorescein circulation time may exceed 30 seconds when the mean blood pressure is high and the general state of the animal is fair to all appearance.

The difference between the cyanide and fluorescein circulation times cannot be accounted for by the longer distance involved in the measurement by the fluorescein technique, for the control circulation time by this method is at most only 1 or 2 seconds longer than when cyanide is used. The discrepancy between the two methods during shock is mainly related to the fact that in the measurement by fluorescein the end-point is taken when the conjunctival mucosa is suddenly and fully fluorescent. This, in turn, is dependent upon the pressure and upon the condition and caliber of the arteriolar and capillary vessels involved (see also Lange and Boyd, 1944). In animals in which the upper part of the body including the head had been previously sympathectomized, the fluorescein circulation time is maintained at a constantly increased value after trauma and gives no clue as to the eventual fate of the animal (Wang, Painter, and Overman, 1946). In such an animal the cyanide and fluorescein measurements follow identical patterns. In the unoperated animal the fluorescein

TABLE III

Comparison of Fluorescein Circulation Time (F) in Seconds, and Oxygen Content in Jugular Venous Blood (V) and Difference in Oxygen Content in Arterial and Jugular Venous Blood (A-V) in Volumes Per Cent

Dog No. and sex	Control	No. of hrs. after trauma					Duration of survival
		1–2	2–3	3–4	4–5	5–6	
Deaths							
							hrs
5 ♀	(F) 15	42	52	98			3.7
	(V) 12	4		2			
	(A-V) 7	17		18			
17 ♂	(F) 9	21		34			6.0
	(V) 14	4		4			
	(A-V) 3	11		12			
18 ♂	(F) 15		42		46		6.0
	(V) 11		3		4		
	(A-V) 9		17		16		
Survivals							
24 ♀	(F) 11	22		16		16	
	(V) 15	10		16			
	(A-V) 5	11		5			
26 ♀	(F) 14	38	38		38	38	See discussion
	(V) 11	9	6		6		
	(A-V) 7	12	14		14		
27 ♂	(F) 14	30	26	25		21	
	(V) 11	7	6	7			
	(A-V) 7	13	13	11			
28 ♂	(F) 13	19	24	40		52	See discussion
	(V) 9	4	5	3		4	
	(A-V) 8	14	13	15		14	
29 ♂	(F) 16	29		30	28	28	
	(V) 8	8		6	6	6	
	(A-V) 9	13		13	13	13	
30 ♂	(F) 13	53	38	35		34	
	(V) 16	10	10	8		7	
	(A-V) 3	9	10	11		12	
31 ♀	(F) 9	29		18	21	19	
	(V) 9	7		5	4	4	
	(A-V) 8	9		12	14	14	

circulation time method has the advantage, therefore, of reflecting constriction and failure in the arteriolar and capillary circulations, which play a significant rôle in the manifestations of tissue anoxia and shock.

Although it is generally true that a circulation time of over 30 seconds by the fluorescein method denotes a grave prognosis, it is hazardous to use a single determination as a criterion for forecasting the fate of the traumatized animal. Indeed, several of our dogs survived following trauma with a circulation time longer than 30 seconds, and four of the dogs died which had an initial posttraumatic circulation time of 30 seconds or less. Nevertheless, for practical purposes, it would seem wise to institute preventive therapy whenever the circulation time is found to be above the normal range. However, in an experimental study in which the prognosis in the individual preparation is desired, at least two determinations separated by an interval of 1 hour or longer are necessary.

An analysis of the data presented in Table I shows that if the second circulation time is not only longer than the first, but also over 30 seconds, the chance of survival is small. Indeed, if the first posttraumatic circulation time is greater than 30 seconds and the second is longer than the first, the untreated animal will always die. In many instances the circulation time will increase progressively up to 50 to 90 seconds or more about one-half hour before death. If, on the other hand, the first circulation time is less than 30 seconds and the second is shorter than the first, the animal will invariably survive. In fact, a decreasing circulation time, regardless of the value of the initial posttraumatic reading, is generally indicative of survival.

The data as tabulated (Table I) show an occasional exception to these general rules. For instance, dog 28 survived although the circulation time showed a progressive increase which 6 hours after injury amounted to 52 seconds. This dog had a very low residual blood volume (65 cc. per kilo of body weight) (Wang, Overman, Fertig, Root, and Gregersen, 1946). After trauma the mean blood pressure declined from 98 to 52 mm. Hg and the heart rate increased to 240 beats per minute. The arterial-jugular venous oxygen difference was high; the venous oxygen content was low. Not only did the circulation time mislead us, but also none of the other criteria used suggested that the dog would survive.

On the other hand, a decreasing circulation time may not always be indicative of survival. Thus, in dog 14 (Table I) the second circulation time was 28 seconds, 4 seconds shorter than the first determination. The blood pressure was maintained at 95 mm. Hg throughout the period of observation. Nevertheless, a few minutes after the second fluorescein injection, the condition of the animal became suddenly worse and the blood pressure fell to 78 mm. Hg. The dog was taken from the cradle and after 1 hour during which time its condition had failed to improve the circulation time was 95 seconds. This reading correctly forecasts imminent death which occurred a few minutes later.

If, as in several experiments, the first two circulation times are not sig-

nificantly different, it is necessary to make a third determination in order to assess the condition of the animal. However, as previously stated, it is our impression that if the first two circulation times are less than 30 seconds, the animal has a good chance of survival. In one animal (dog 26) the circulation time remained at 38 seconds and the dog survived. In this case neither the circulation time nor the A-V oxygen difference which remained at 14 volume per cent were of any prognostic aid (see Table III).

It is evident that in many of our experiments the change in fluorescein circulation time accurately predicted eventual recovery or death at a time when the blood pressure and heart rate remained at a plateau level for 3 to 4 hours (Fig. 1). When used in this way, the prognosis can be made early in the course of impending shock. For instance, if, before treatment, the second circulation time is found to be more prolonged than the first and both are over 30 seconds, then the animal would not be expected to live, and the efficacy of the therapy may be safely judged. It should, perhaps, be pointed out that the above criteria were established while keeping the animal in the cradle for 6 hours without any manipulation except to take a few cubic centimeters of blood for plasma volume determination.

In experiments not reported here (Wang and Painter, 1943) we have seen that transfusion is followed for a brief period by a definite improvement in the fluorescein circulation time even in animals that may eventually die. In other words, a single short circulation time following therapy indicates a transient recovery, but this cannot be taken to forecast survival until the trend of change is again determined by subsequent readings. It should also be pointed out that simple hemorrhage with a comparable or a more severe degree of blood loss as indicated by the residual blood volume does not markedly increase the circulation time. The value is generally less than 30 seconds, despite the low blood pressure (Wang, Painter, and Overman, 1946). This indicates, perhaps, that the peripheral arterial system in animals suffering from simple hemorrhage is not maximally constricted (Frank, Altschule, and Zamcheck, 1945), or the blood flow is not as severely impaired as that in animals subjected to muscle trauma (Wang, Overman, Fertig, Root, and Gregersen, 1946).

Since the important manifestations of shock are related to tissue anoxia (see Root, Walcott and Gregersen, 1946) the fate of the traumatized animal should be determined by the improvement or impairment of the blood flow to important organs, such as the brain, the heart, or liver. Unfortunately, we do not have a simple and reliable means for the direct determination of blood flow in unanesthetized normal and traumatized animals. If the capacity of the tissues to use oxygen is unchanged, it would appear that the A-V oxygen difference should be closely related to the volume flow of blood. In several experiments, however, the A-V oxygen differences progressively increased during the first 2 hours after trauma, and yet the fluorescein circulation time indicated

improvement and the animal recovered. The discrepancy between the circulation time and A-V oxygen difference can be explained by assuming that the metabolism is initially depressed for some time after ether and muscle trauma. If this occurs, the venous oxygen level may be falsely high; the A-V oxygen difference correspondingly low. In such a situation the circulation time measurements may be a more accurate index of the animals' condition than the determinations of the A-V oxygen differences.

There are a number of objective methods for the determination of circulation time (Bellis, 1943). Since the circuit measured by each method is different, it is important to select a method that includes a circuit having all the essential parts of the circulatory apparatus. We believe that fluorescein is the method of choice. It is true that it has certain drawbacks: first, the end-point, though objective, is dependent upon the experience and judgment of the observer and is not recorded by an instrument, and second, fluorescein does not disappear rapidly from the conjunctival mucosa under shock conditions so that it is increasingly difficult to decide on the end-point in subsequent determinations.[1] However, in animals in shock even at the time of the very first fluorescein injection, the end-point is not sharp, presumably because the blood flow in the capillaries is already slowed as a consequence of loss of blood and vasoconstriction, and the fluorescein concentration in the tissue must rise to a considerable value before a decision as to the end-point can be reached. In other words, the end-point depends not only on the speed of blood flow but also, what is more important, on the quantity of the blood in the area.

SUMMARY AND CONCLUSIONS

Repeated determinations of the circulation time by the fluorescein method were made in normal and shocked dogs. In normal animals the circulation time ranges from 9 to 16 seconds with an average of 12.6 seconds. In traumatic shock the circulation time is invariably prolonged.

For prognosis in the traumatized animal two determinations of fluorescein circulation time separated by an interval of 1 hour are essential. If the second circulation time is longer than the first and both are over 30 seconds, the animal will not survive without therapy. On the other hand, if the second circulation time is below 25 seconds or is considerably shorter than the first, the prognosis is good. In many of these experiments the change in circulation time appeared to be the earliest index of eventual recovery or death. It gave a clue to the fate of the animal when no decisive judgment could be made from the blood pressure and heart rate.

In three dogs the cyanide and fluorescein circulation times were compared

[1] These drawbacks may be remedied by the use of a Dermofluorometer (Lange and Kremer, 1943). The residual fluorescein is eliminated and the rate of diffusion following each fluorescein injection is then recorded (personal communication from Dr. Lange).

during shock. It was found that the cyanide circulation time, though increased in shock, remained at a fairly constant value while over the same period the fluorescein circulation time showed progressive changes. This discrepancy between the cyanide and fluorescein methods may be explained by the fact that the former does not include the minute peripheral systemic circulation. Since the study of shock is concerned with tissue anoxia and is primarily a phenomenon of the failure of the peripheral circulation, it is important to choose procedures such as the fluorescein method as a measure of the condition of the peripheral vascular system.

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