UR-401

THE RELATIONSHIP OF THERMALLY INDUCED
HEMOGLOBINEMIA TO VOLUME OF SKIN BURN

by:

J. L. Lyon
A. J. Emery, Jr.
H. E. Pearse
T. P. Davis

THE UNIVERSITY OF ROCHESTER
ATOMIC ENERGY PROJECT
ROCHESTER, NEW YORK

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

U N C L A S S I F I E D

- 1 -

UR-401

Health and Biology

THE UNIVERSITY OF ROCHESTER
Atomic Energy Project
P. O. Box 287, Station 3
Rochester 20, New York

* * *

Contract W-7401-eng-49 between the U. S. Atomic
Energy Commission and the University of Rochester,
administered by the Department of Radiation Biology
of the School of Medicine and Dentistry.

THE RELATIONSHIP OF THERMALLY INDUCED
HEMOGLOBINEMIA TO VOLUME OF SKIN BURN

This document is
PUBLICLY RELEASABLE

by:

Hugh Kinser
Authorizing Official
Date: 3/15/10

J. L. Lyon
A. J. Emery, Jr.
H. E. Pearse
T. P. Davis

Division: Special Programs

Division Head: H. A. Blair

Section: Flash Burns

Section Head: H. D. Kingsley

Date Completed: 5/27/55

Date of Issue: 6/30/55

U N C L A S S I F I E D

THE RELATIONSHIP OF THERMALLY INDUCED
HEMOGLOBINEMIA TO VOLUME OF SKIN BURN.

By

J. L. Lyon
A. J. Emery, Jr.
T. P. Davis

ABSTRACT

The precept, that the mass of an animal's circulating hemoglobin is proportional to surface area, has been used to correlate levels of thermally induced hemoglobinemia with burn severity. Anesthetized pigs were burned over varying portions of their surface area, at graded exposure times in water at 80° C. Sections of skin, stained by various methods, were assessed for burn depth. Plasma samples were drawn at specified intervals post burn, and the hemoglobin content related to depth and surface area burned.

A method has been evolved whereby the average depth of a burn may be estimated if the surface area relationships and post burn hemoglobin concentration are known.

INTRODUCTION

Depth and surface area burned are the fundamental parameters of thermal injury to the skin. At the present time only the surface area burned can be accurately measured. In the human subject, particularly, no good method of assessment of depth is available. Biopsies obviously are impractical, for even if they were taken, only information regarding the site of biopsy would result. The measurement of volume of tissue burned offers an avenue of approach to the problem of obtaining the unknown factor in burn severity, i.e., depth.

Hemoglobinemia is known to occur following most burns of significant extent. The bulk of the erythrolysis necessary to release free hemoglobin in the plasma is most probably produced at the time of the burn episode. Other things being equal it is likely that the quantity of red blood cells destroyed will depend upon the surface area exposed and the depth at which hemolytic temperatures are attained. A few reports (4, 8, 2) of actual measurement of circulating hemoglobin in the early post burn period have appeared, but systematic quantitation of this substance, in terms of burn severity, has never been attempted.

This experiment was designed in such a way that known volumes of pig skin were burned with water at 80° C. Blood samples drawn at specified times following each burn, have been analyzed for their plasma hemoglobin content. The observed degrees of plasma hemolysis have been correlated with the known volumes of burned tissue, and a method has been evolved that permits the estimation of the average depth of a burn from the plasma hemoglobin concentration resulting from the thermal exposure.

METHODS

I Physical

Large area, circumferential burns were produced by dipping anesthetized pigs into hot water contained in a galvanized can, 24 by 36 inches. Water was circulated rapidly through the container by placing the inflow hose at the bottom and allowing the water to freely overflow the brim. The water temperature for the entire study was constant at 80° C., and because of the large volume of water flowing through the container, the water temperature never varied more than 2 degrees during any given burn procedure. Suspension of the animals was carried out with a double loop rope harness. The cephalad loop was placed around the animal's chest just behind the fore-limbs, and the caudal loop around the body just proximal to hind-limbs. The upper loop was then attached to a chain from an overhead single pulley and the lower loop secured to a chain from a pulley in the bottom of the can. With this method, the operator had complete control of the vertical movement of the animal, and immersion, as well as removal from the water, could be done in less than 0.5 sec.

Protection of the skin surface was accomplished by wrapping two thicknesses of 1/2 inch sponge rubber around the animal's body, so that only the area desired for burning was left exposed. The rear hoofs of the animals were wrapped with several thicknesses of gauze, and then covered with a 1 inch penrose rubber drain. This provided satisfactory insulation of the hoofs and allowed the animals to be ambulatory as soon as the anesthesia wore off.

II Biological

A. Animals

Chester White, male and female, pigs, weighing from 6 to 23 kg., were used for this study. They were anesthetized 1/2 hour before burning with Dial in

urea-urethane, Ciba, in amounts of 40 milligrams per kilogram of body weight, given intraperitoneally. The total surface area was determined in accordance with the values established by Kingsley (3) which follow the formula:

$$S. A. = .1 W^{.62}$$

S. A. = Surface area in square meters

W. = Weight in kilograms

The percent that various portions of the pigs skin surface are of the total area have been found to correspond quite well to the "Rules of 5". This is an adaptation to the pig of Wallace's (9) "Rules of 9" for humans, and is represented in Fig. 1, where each number indicates the percent that particular area is of the total. The "Rules of 5" was used to determine the amount of skin to be exposed for burning in this experiment.

Initially the surface area burned was kept constant at 60% with exposure times of 10, 20, 30, and 40 sec. Five replications were used for each time-area combination or a total of 20 pigs. As a second step both the area and the times of exposure were varied. The areas selected were 45, 30 and 15% with exposure times of 20, 30, and 40 sec., yielding nine combinations. Three animals were used for each of these time-area groupings, or a total of 27 pigs. Selection of animals and the order of burning was completely randomized. One animal was prepared for burning and carried through the entire procedure but was not burned. This served as a control for the method.

Each animal was returned to its cage as soon as all samples were drawn and no therapy was given. They were allowed to take food and water ad lib.

B. Biopsies

Skin biopsies were secured at 10 minutes, 24 hours, and when survival permitted, at 72 hours post burn. The 10 minute specimens were stained with

Fig. 1

"Rules of 5" For Pigs

hematoxylin and eosin, as well as the orange G, aniline blue method of Ross and Walker (5). The 24 and 72 hour specimens were stained only with hematoxylin and eosin.

Good agreement for depth of burn existed for the different methods of staining and the varied time intervals post burn that the biopsies were secured. With practice, the 10 minute specimens stained with hematoxylin and eosin could be read for depth of injury. The 24 and 72 hour sections were somewhat more easily interpreted due to collagen denaturation and the formation of the pyogenic membrane. The orange G, aniline blue method was used only for sections obtained immediately post burn.

All depth measurements were taken from the surface of the epidermis and may be summarized as follows, for the exposure times used:

Exposure Time	Water Temperature	Depth of Damage
10 Sec.	80° C.	.2 mm $\pm$.1 mm
20 Sec.	80° C.	.5 mm $\pm$.1 mm
30 Sec.	80° C.	.9 mm $\pm$.1 mm
40 Sec.	80° C.	1.4 mm $\pm$.1 mm

A depth of 1.4 millimeters has been adopted to represent the average thickness of skin for pigs in the size range used in this experiment. The experience in this laboratory indicates that the depth of the skin may vary from 1.3 millimeters on the abdomen to 1.6 millimeters on the animals' back. It will be seen that a 40 second exposure to water at 80° C. produces a burn at least 1.4 millimeters deep. This represents a full thickness burn in terms of the average depth of pig skin. It should be mentioned that some of these burns may have extended a short distance into the fat, however, the exact

TABLE I
EXPERIMENTAL DATA FOR BURNED PIGS.

Exposure Time	Surface Area Burned	Pig Wt. Kg.	Control	Mgm. % Plasma Hemoglobin as Hemolysis for Time in Minutes Post Burn						Death Post Burn	Total S. A. cm ²	S. A. Burned cm ²	Av. Depth Burned Skin cm	Vol. of Skin Burned cm ³	V _B S.A.T	
				30	60	120	200	270	340							
10 Sec.	60%	11.6	60	35	-	56	56	49	67	7 Days	4580	2750	.02	55	.012	
		12.7	50	66	-	48	28	45	71	7 "	4930	2960	.02	59	.012	
		13.7	55	131	-	147	110	127	83	7 "	5080	3050	.02	61	.012	
		20.1	35	65	-	86	-	42	25	7 "	6750	4050	.02	81	.012	
		16.8	20	51	-	76	123	118	134	7 "	5800	3480	.02	70	.012	
Numerical Average				69	-	83	79	76	77							
20 Sec.	60%	11.2	52	114	288	148	-	166	96	7 Days	4520	2720	.05	136	.030	
		9.7	87	203	242	166	260	269	218	50 Hours	4100	2460	.05	123	.030	
		20.6	58	161	-	186	211	154	-	7 Days	6800	4080	.05	205	.030	
		15.2	-	186	185	227	231	175	147	7 "	5400	3250	.05	162	.030	
		11.6	4.0	140	160	148	134	127	99	7 "	4500	2700	.05	135	.030	
Numerical Averages				161	216	175	209	178	106							
30 Sec.	60%	13.7	40	436	370	450	314	316	261	40 Hours	5100	3050	.09	275	.054	
		15.7	40	565	505	449	412	381	348	7 Days	5550	3320	.09	300	.054	
		19.1	65	415	385	382	318	306	200	3 "	6275	3770	.09	340	.054	
		19.9	73	336	-	570	520	660	-	7 Hours	6350	3810	.09	390	.054	
		16.5	28	452	352	-	-	-	-	3 Days	5800	3480	.09	310	.054	
Numerical Average				441	403	465	391	416	269							
40 Sec.	60%	14.6	59	794	821	704	-	564	422	48 Hours	5025	3020	.14	420	.083	
		16.7	60	615	690	750	793	709	540	36 "	5780	3460	.14	480	.083	
		10.7	50	492	610	495	608	378	344	36 "	4370	2620	.14	370	.083	
		15.0	27	870	847	776	599	533	-	24 "	5400	3250	.14	450	.083	
		Numerical Average				693	742	681	667	546	435					
20 Sec.	45%	11.15	40	200	254	250	292	292	203	3 Days	4400	1980	.05	99	.023	
		11.7	45	133	166	172	-	117	161	55 Hours	4570	2060	.05	100	.023	
		7.9	60	111	250	211	242	-	-	56 "	3600	1620	.05	81	.023	
		Numerical Average				149	223	211	267	205	182					
		30 Sec.	45%	14.9	70	146	237	294	282	206	222	48 Hours	5320	2400	.09	220
7.8	-			423	554	576	563	450	364	36 "	3550	1600	.09	140	.041	
13.4	40			135	227	182	251	180	218	28 "	4950	2230	.09	200	.041	
Numerical Average				235	339	352	365	295	268							
40 Sec.	45%			6.7	30	438	348	293	368	347	284	20 Hours	3250	1460	.14	200
		17.4	45	360	610	455	532	236	314	40 "	5850	2630	.14	370	.062	
		11.7	-	244	250	250	281	240	271	30 "	4560	2050	.14	290	.062	
		Numerical Average				347	423	366	393	278	289					
		20 Sec.	30%	7.7	55	109	-	140	107	120	111	20 Hours	3550	1060	.05	53
8.7	-			148	-	169	233	171	125	40 "	4070	1220	.05	61	.015	
10.6	90			121	180	203	-	197	148	33 "	4300	1290	.05	64	.015	
Numerical Average				126	180	171	170	163	128							
30 Sec.	30%			8.5		Died 2 Hours Post Burn						2 Hours	3780	1140	.09	100
		8.1	30	259	-	279	217	196	231	10 "	3650	1100	.09	99	.026	
		6.4	20	242	327	306	180	251	210	20 "	3160	950	.09	86	.026	
		Numerical Average				251	327	293	199	224	221					
		40 Sec.	30%	7.65	40	250	270	-	240	190	160	9 Hours	3500	1050	.14	150
9.5	45			175	279	245	238	211	185	18 "	4020	1210	.14	170	.043	
7.2	-			389	370	351	292	147	154	40 "	3400	1020	.14	140	.043	
Numerical Average				271	306	298	290	249	166							
20 Sec.	15%			10.2	30	181	133	119	196	161	105	30 Hours	4200	630	.05	32
		13.3	25	-	76	148	140	101	117	7 Days	4950	740	.05	37	.008	
		11.1	40	108	173	138	151	107	147	44 Hours	4430	670	.05	34	.008	
		Numerical Average				145	128	135	162	123	123					
		30 Sec.	15%	7.5	20	203	159	178	203	294	156	30 Hours	3500	530	.09	48
13.9	-			175	189	204	235	155	279	55 "	5100	770	.09	69	.014	
11.3	-			95	300	250	210	235	132	33 "	4450	670	.09	60	.014	
Numerical Average				158	216	211	216	228	189							
40 Sec.	15%			13.5	30	148	278	336	353	198	150	30 Hours	5000	750	.14	110
		7.4	46	255	300	349	297	200	300	22 "	3450	520	.14	73	.022	
		7.9	29	230	268	251	250	190	250	6 Days	3600	540	.14	76	.022	
		Numerical Average				211	282	312	300	196	233					

Fig. 2
Concentration of Hgb. in Mgm. % vs. Time Post Burn for Burns
of 60% Surface Area, H₂O @ 80° C.

Fig. 4

Concentration of Hgb. in mgm. % vs. Time Post

Burn for Burns of 30% Surface Area, H₂O @ 80° C.

Figure 2 is plotted for 60% surface area exposed, Figure 3 for 45%, Figure 4 for 30%, and Figure 5 for 15%. The control level of 45 milligrams percent plasma hemoglobin is the lower curve in each diagram, and was obtained by averaging all of the pre-burn control values in Table I. It indicates a basal reading for the method used, and is not to be construed as an absolute value for the normal plasma hemoglobin content in pigs.

Examination of Figures 2 - 5 indicates that hemoglobin levels rise sharply to 30 minutes followed by a more gradual leveling off to 120 minutes post burn, and then are characterized by a slow decline to 340 minutes. This is the general contour observed for all the curves and would seem to indicate that either hemolysis continues after the heat is removed, or that the routes of excretion cannot initially keep pace with the rapid movement of hemoglobin into plasma. The peak plateau of the majority of the curves occurs around 120 minutes and the hemoglobin values at this time interval have been used to interpret the data. It is possible that samples obtained at times greater than two hours post burn may be extrapolated back to this time interval. It should be noted that so little difference was found between the degree of hemoglobinemia for 30% and 15% burns, that with the method used it is difficult to distinguish one from the other. From this it is apparent that the experimental results are probably valid only for burns of 30% surface area or greater.

Figures 6 and 7 have been constructed to illustrate some relationships of the experimental data. In Figure 6 the depth of burn in centimeters has been plotted against the exposure time in seconds. The range of depth for each exposure time is shown by I symbols. The shape of this curve may indicate the dependence upon time for the establishment of a temperature gradient through the skin.

In Figure 7, milligrams percent of plasma hemoglobin has been plotted against exposure time in seconds for each of the four surface areas burned.

Fig. 6

Fig. 7

Mgm. % Plasma Hgb., 2 Hours Post Burn, vs. Exposure Time
(Sec.) for Each Percent Surface Area Burned.

Note that the curve for 60% burns rises more sharply with increased exposure time while the curves for smaller surface areas tend to assume a more linear relationship. This may indicate that for larger area burns at longer exposure times, the factor of blood flow beneath the heated area becomes important. In other words, as the exposure time is prolonged, more undamaged red blood cells are made available for heat alteration at the burn site. In addition, the total vascularity, as well as vessel size, increase somewhat as the deeper layers of the dermis are reached. This would also tend to expose more blood to the erythrolytic action of heat as the exposure time is prolonged. These two factors probably account for higher plasma hemoglobin levels than would obtain if the blood were stationary and hemolysis were a function of depth only. As the surface area burned is decreased, the flattening of the curves may indicate that these two phenomena have less influence on the degree of hemoglobinemia.

DISCUSSION

Understanding the volume concept of burn severity is based upon the knowledge that plasma volume and total circulating hemoglobin, with constant hematocrit, are proportional to the skin surface area rather than to body weight. Rowntree (6, 7) established this for humans and it has been confirmed in this laboratory for pigs employing the Evans Blue Dye technique. The extension of this relationship to apply to the volume of skin may be stated as follows: assuming a constant average depth for normal skin, the total skin volume will be proportional to the total surface area and therefore to the plasma volume and to the total circulating hemoglobin. If one now assumes that the number of red blood cells exposed to heat lysis varies directly with the depth and surface area of a burn; then it is reasonable to suppose that the mass of hemoglobin released into the plasma would also be proportion to this burned volume.

Fig. 8. Two hour plasma hgb. concentration in mgm. % vs. volume of burn divided by total surface for each experimental burn. The solid line is a least squares fit to the experimental points; the dashed lines are the 95% confidence limits.

It follows from the above that, if the mass of heat released hemoglobin is expressed as units per 100 cc. of plasma; then this value will be proportional to the burned skin volume divided by the total surface area of the animal in question. Thus:

$$[\text{Hb}]_{\text{pl}} = \frac{\text{S. V.}_B}{\text{S. A.}_T}$$

where: $[\text{Hb}]_{\text{pl}}$ = plasma hemoglobin concentration in mgm %
 S. V._B = skin volume burned in cm^3
 S. A._T = total skin surface area in cm^2

Introducing the proportionality constants:

$$[\text{Hb}]_{\text{pl}} = a + k \frac{\text{S. V.}_B}{\text{S. A.}_T} \quad (1)$$

where: a = control level of hemoglobinemia in mgm % plasma for unburned pigs
 k = the proportionality constant relating plasma volume and surface area

The fractions obtained for skin volume burned divided by total surface area, for each experimental burn, are presented in the final column of Table I. In Fig. 8 the two hour plasma hemoglobin concentrations are plotted against the burn volume, surface area fractions for each burn studied. The solid line is a least squares fit through the experimental points and from it the proportionality constant, k , has been calculated to be 6.84×10^3 . The control level of hemoglobinemia for unburned pigs, (a) in equation 1, was found to be 49.2 mgm %. The two dotted lines indicate the 95% limits of confidence for a single plasma hemoglobin sample drawn for one specific burn.

Equation #1 may now be solved for depth of burn. Let

S. A._B = surface area burned in cm^2
 D_B = average depth of burn in cm

Fig. 9. Nomogram relating percent surface area burned and mgm. % plasma hgb. to average depth of burn.

substituting in equation #1:

$$[\text{Hb}] \quad \text{pl} = a + k \frac{(\text{S. A.}_B) (D_B)}{\text{S. A.}_T}$$

solving for depth:

$$D_B = \text{S. A.}_T \frac{([\text{Hb}] \text{ pl} - a)}{(k) (\text{S. A.}_B)} \quad (2)$$

Thus, a mathematical expression evolves by which the average depth of burn may be calculated if the surface areas relationships and plasma hemoglobin concentration are known.

In order to more simply demonstrate the concept just discussed, the nomogram in Figure 9 has been constructed. The left-hand column represents the percent of the total surface area burned, the middle column is the average depth of burn, and the final column indicates the two hour post burn hemoglobin concentration in mgms. per 100 cc. of plasma. The point at which a line drawn between the percent surface area burned and the mgms. percent of plasma hemoglobin intersects the middle scale, gives the average depth of the burn. Plasma hemoglobin levels have been taken from the mean least squares curve of Figure 8.

For the purpose of illustrating the potential usefulness of these relationships, assume an animal burned over 50% of its surface and manifesting a two hour hemoglobin level of 120 mgms. percent. The average depth of its burn will be .02 cm., a relatively shallow burn. Another animal with a 50% burn showing a two hour hemoglobin level of 600 mgm. percent will have a burn of .14 cm. average depth, which is a full thickness lesion as gauged by the criterion employed in this study. It is evident that these two burns, similar in gross appearance, and identical in percent of surface area burned, are quite different from the volume standpoint.

Obviously this material, obtained for pigs, cannot be directly transferred to the human burn casualty. However, it is felt that the volume concept of burn severity is fundamentally sound and from this standpoint may be worthy of further investigation. It is hoped that other workers interested in the large area burn problem will test the basic hypothesis here presented.

SUMMARY AND CONCLUSIONS

1. Methods have been described for producing large area hot water burns in pigs and for estimating surface areas by the "Rules of 5".
2. Thermally induced hemoglobinemia has been correlated with depth and surface area of burn.
3. A volume concept of burn severity has been suggested, by means of which, an equation, expressing depth as a function of surface area and post burn hemoglobinemia, has been evolved.

BIBLIOGRAPHY

1. Evelyn, K. A., and Malloy, H., Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulphemoglobin in a Single Sample of Blood, J. Biol. Chem., 126:655, (1938).
2. Hobson, L. B., and Harvey, T. S., Medical Division Report Number 113, Flame Attack Study VI. Studies on Goats Directly Exposed to Flame of Petroleum Fuels Fired from Flame Throwers, Chemical Warfare Department Technical Report, Washington, D. C., (1947).
3. Kingsley, H. D., Personal communication.
4. More, F. D., Peacock, W. C., Blakely, E., and Cope, O., The Anemia of Thermal Burns, Annals of Surgery, 124:811, (1946).
5. Ross, O. A., and Walker, C. J., Histochemical Studies of Thermal Injury on Rat Skin, Proceedings of the Society of Experimental Biology and Medicine, 379, (1953).
6. Rowntree, L. G., The Water Balance of the Body, Physiological Review, 2:116, (1922).
7. Ibid, The Volume of the Blood and Plasma in Health and Disease, Saunders, Philadelphia.
8. Shen, S. C., and Ham, T. H., Studies on the Destruction of Red Blood Cells, New England Journal of Medicine, 229:701, (1943).
9. Wallace, A. B., Surface Assessment of Burns, Practioner, 170:109, (1953).