The Biochemical Response to Injury

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FOREWORD

The key function of the Council for International Organizations of Medical Sciences (C.I.O.M.S.) is to provide the mechanism by which the voluntary organizations of scientists in every medical discipline in every country in the world can be brought into co-operative relationship. The organization of symposia is one way of attaining this objective, and since its creation in 1949 the C.I.O.M.S. has arranged a number of these meetings. Most were organized in relation to one of the great international congresses, others were held independently.

The present symposium on 'The Biochemical Response to Injury' was organized with the co-operation of the International Union of Biochemistry and was held immediately after the 4th International Congress of Biochemistry. The meeting took place at the Semmering from September 7th to 12th, 1958, under the chairmanship of Dr D. P. Cuthbertson. Dr H. B. Stoner, who was the mainspring of the idea of holding such a symposium, acted in the capacity of scientific secretary.

It is a pleasant duty to record the Council's gratitude to Dr Cuthbertson and to Dr Stoner for the magnificent way the meeting was conducted and organized. Much praise should go to Dr F. G. Wewalka who made the local arrangements for the symposium. That the meeting was held at all is due to a grant from the Wellcome Trust. May the Trustees and their Scientific Secretary find here an expression of the Council's indebtedness.

The work of editing this monograph and the difficult task of recording the main aspects of the discussion has fallen to Dr H. B. Stoner. In this he has had considerable assistance from his colleague, Mr C. J. Threlfall. Frau S. Pichler and Miss B. A. V. Dunford must also be thanked for their secretarial assistance. Dr Stoner may justly feel pleased with the result of his efforts, for the proceedings of this symposium form a substantial contribution to new knowledge and an invaluable synopsis of current thought on the biochemical response to injury.

J. F. DELAFRESNAYE

ABBREVIATIONS

PC	phosphocreatine
ATP	adenosine triphosphate
ADP	adenosine diphosphate
AMP	adenosine monophosphate
IMP	inosine monophosphate
GTP	guanosine triphosphate
UTP	uridine triphosphate
ΔP7′	the amount of inorganic phosphorus liberated by 7 min. hydrolysis in N acid at 100°
~P	'energy-rich' phosphate (Lipmann, 1941)
P/O	the ratio of the atoms of phosphorus esterified into ATP to the atoms of oxygen consumed
DPN	diphosphopyridine nucleotide, oxidized form
DPNH	diphosphopyridine nucleotide, reduced form
TPN	triphosphopyridine nucleotide, oxidized form
TPNH	triphosphopyridine nucleotide, reduced form
CoA	coenzyme A
DNA	deoxyribose nucleic acid
RNA	ribose nucleic acid
G-1-P	glucose-1-phosphate
G-6-P	glucose-6-phosphate
F-6-P	fructose-6-phosphate
HDP	fructose-1-6-diphosphate
PG	phosphoglyceric acid
αGP	α-glycerophosphate
βGP	β-glycerophosphate
NPN	non-protein nitrogen
ACTH	pituitary adrenocorticotrophic hormone
17 OHCS	17 hydroxycorticosteroids
5-HT	5-hydroxytryptamine
48/80	compound 48/80, a condensation product of *p*-methoxy-phenethyl-methylamine with formaldehyde
ECG	electrocardiogram
S.D.	standard deviation
S.E.	standard error

All temperatures are expressed in degrees Centigrade.

CONTENTS

INTRODUCTION

D. P. CUTHBERTSON

While in the more recent past the main stimulus to the study of the effects of injury has undoubtedly been derived from war, the older literature contains interesting descriptions of some of the events following trauma. In *A Treatise on the Blood, Inflammation and Gunshot Wounds* published in 1794, John Hunter wrote: 'There is a circumstance attending accidental injury which does not belong to disease, namely, that the injury done, has in all cases a tendency to produce both the disposition and the means of cure.' The necessity for a wide concept of the reaction to injury was also stressed by Malcolm in his classical description of *The Physiology of Death from Traumatic Fever* (1893) when he stated that 'shock is more a part of phenomena caused by injury, whether surgical or otherwise, than a complication thereof'.

World War I saw the emergence of the concept of primary and secondary wound shock – primary akin to syncope, and secondary a widespread depression in functional activity of many organs and tissues remote from the site of serious injury. But these phenomena were in part already known to Hunter. Longmore writing in 1877 described how in very severe wounds 'pain is deadened either by annihilation of function and sensation of the parts wounded, or by the state of stupor into which the shock of injury has thrown the patient ... In most cases the early pain, even if severe, is evanescent, and is followed by a certain amount of numbness. As a rule the graver the injury the greater and more persistent is the amount of shock'. Towards the end of the nineteenth century Crile (1897) was able to put the subject on to an experimental basis.

In 1910 Dale and Laidlaw had pointed out the reduction of blood pressure which results from the injection of histamine into cats. Along with Richards they investigated the phenomenon more fully during the later stages of the first world war and it was their view that the effects bore a closer resemblance to those occurring during wound shock than was at first expected. These workers found that the low pressure resulting from the administration of histamine was apparently not due to a relaxation of the arteries but to a dilatation

of the capillaries with, in addition, an escape of plasma into the tissue spaces – a condition which Malcolm (1905) had surmised from clinical observation. During World War I the French observer Quénu and his colleagues came to the view that everything that favoured absorption at the site of injury favoured the development of secondary shock. Thus arose the toxaemic theory. The bacteriological studies of Almroth Wright and Colebrook on gas gangrene added further definition to toxaemic factors of bacterial origin. Bayliss and Cannon (1919) then pursued the idea of a toxic substance developed and liberated from injured tissues. Readers of these present proceedings will note that it now seems likely that substances liberated from the damaged tissue may cause increased capillary permeability and produce some of the effects of acute inflammation.

While Duval and Grigaut (1918) had noted that there was an increase of the blood non-protein nitrogen (both urea and non-urea NPN) and that this was related to the degree of tissue damage, and while some cursory analyses of the urine made at that time indicated a rise in urinary nitrogen, little further study of the biochemical effects of physical injury took place between the two world wars, apart from efforts in one or two places. The uneasy years since World War II have seen an extension of the scope of such investigations, particularly as newer experimental techniques have become available. Thus the sequence of events which constitutes the response to injury is gradually being elucidated as different facets of the picture are being obtained through the use of these new approaches. How far the response is the resultant of a chain reaction and how far it arises from a collateral series of such reactions is still a matter of investigation and conjecture.

As this store of accumulating knowledge requires review from time to time, it seemed wise that a period of time should be set aside at this stage so that those most active in this field might come together to see how far the events they were investigating could be pieced together – since out of it there must come a synthesis – and also how far new light could be shed from the results of other and parallel lines of approach, and also where the main gaps lay. An understanding of the response to injury is basic to our understanding of medicine as a whole. The formulation of the stress-concept by Selye about 1936 and its subsequent elaboration by him into the general adaptation syndrome has undoubtedly provided such a unifying concept. But in its present state, it seems to have outstripped

the experimental evidence and by its very over-unification has tended to inhibit further research. Claude Bernard was only too conscious of the dangers of this when in his *Introduction to the Study of Experimental Medicine* in 1865 he wrote: '... the ambition to explain prematurely at one step the whole of the disease ... one loses sight of the patient, one gets a wrong idea of the disease, and by a false application of physiology, experimental medicine is hindered instead of being assisted in its progress.'

The sequence of papers and discussions, which constitutes the substance of this symposium, deals with many of these aspects, some with events arising from mild stimuli, some from more grave effects, and with both the local and the general response. Some deal with events over short periods of time, others continue well into the recovery phase. Some deal with the organism as a living entity, others deal with such aspects as the biochemistry of intracellular enzymes. Some deal with early pathological changes, others with pre-mortem events. In some of the papers cardiovascular changes are described, including the permeability of the capillaries and the behaviour of the red cells and the endogenous substances which can influence them; some with changes in the cerebral tissue. In some experiments the injury is bacterial in origin, in others physical such as that resulting from fracture, haemorrhage or burns, but the physical injury may subsequently be complicated by bacterial injury. The practical aspects of the discoveries are not ignored so that the book should be of great interest to physicians and surgeons as well as to biochemists, physiologists and experimental pathologists.

ELECTROLYTES AND GLYCOGEN IN INJURED TISSUES

FREDERICK A. FUHRMAN

Skin and skeletal muscle are more frequently injured than other tissues. Since they constitute 70 per cent of the mass of the soft tissues of the body, the local changes which take place in them after injury are very important and may be sufficient to produce generalized changes in body composition and function. Fortunately we know more about the electrolyte pattern and its control in these tissues – particularly in muscle – than in any others, and so I shall restrict my discussion to muscle and skin.

ELECTROLYTE COMPOSITION OF MUSCLE AND SKIN

Before discussing the effects of injury on tissue electrolytes and water, I wish to outline the present knowledge of electrolyte distribution in normal tissues. Since we cannot remove the cells from their surroundings, the tissue must first be considered as a whole. Comparison of one normal tissue with another, or normal with abnormal tissue, requires a stable reference base on which to express the analytical data. The constituent chosen as a base should bear a fixed relationship to cell mass and should be unaffected by such variable tissue constituents as water, fat and collagen. Fat-free non-collagen dry tissue (FFNCDT) fulfils most of the requirements for a stable reference base. However, relatively few data exist expressed on this basis. An almost equally stable reference base for most purposes is fat-free dry tissue (FFDT) and I shall use that here. The water, electrolyte and collagen content of rat muscle and skin are compared in Table I. When expressed on the basis of FFDT the two tissues are very different in composition: skin contains more collagen, sodium and chloride and less potassium than muscle. If a correction is made for the 'inert' collagen component, and the potassium concentration expressed per unit FFNCDT, the concentrations in skin and muscle are about equal. Corrections for collagen need not be made when normal muscles are compared or when injured ones are removed within a few days after injury. However, a correction must be made

when comparisons are between normal muscle and injured muscle removed several weeks after injury. These late effects of injury will not be considered in this paper and therefore FFDT is a satisfactory reference base. Expression of data per unit dry tissue is particularly important when water content fluctuates enormously as it may after injury. Attempts to compare normal muscle with oedematous injured muscle on the basis of fresh tissue weight may give misleading or meaningless results.

In this paper parentheses are used to denote g. or mEq. per 100 g./FFDT, and brackets to denote concentrations in mEq. per kg. of

TABLE I

COMPOSITION OF RAT MUSCLE AND SKIN

Skin electrolytes and skin and muscle collagen from Walser and Bodenlos (1954*a*). Muscle electrolytes from Fuhrman and Crismon (1951*b*)

	H_2O *g.*	*Cl mEq.*	*Na mEq.*	*K mEq.*	*Collagen g.*	*K mEq. per 100 g. FFNCDT*
	per 100 g. FFDT					
Muscle	339	6.0	9.5	38.2	4	40
Skin	210	18.7	20.9	6.7	84	42

water. The subscripts e, i, t and s refer to extracellular phase, intracellular phase, total tissue and serum, respectively. Concentrations of ions in plasma ultrafiltrate were calculated using the Gibbs-Donnan ratio

$$[Na]_e/[Na]_s = [K]_e/[K]_s = [Cl]_s/[Cl]_e = 0.96 \qquad (1)$$

The sizes of intracellular and extracellular fluid volumes of tissues may be estimated if one determines the concentration of a constituent which is confined to the extracellular phase. Chloride is often used for this purpose, and recent evidence shows that in normal muscle it is truly extracellular (Cotlove, 1954; cf. Manery, 1954). Conway (1957), however, insists that some intracellular chloride must exist in muscle. In skin, 3 to 4 mEq. of chloride are 'bound' per 100 g. collagen (cf. Walser and Bodenlos, 1954*a*) and suitable correction must be made when extracellular fluid volume is calculated. I shall use the term chloride space in this paper to mean the volume of

water which would contain all the chloride present in tissue in a concentration equal to that in plasma ultrafiltrate.

Chloride space may be calculated as follows:

$$(H_2O)_e^{Cl} = \frac{(Cl)_t}{[Cl]_e} \qquad (2)$$

in which $(H_2O)_e^{Cl}$ = chloride space in g. per 100 g. FFDT, $(Cl)_t$ = chloride content per 100 g. FFDT, and $[Cl]_e$ = chloride concentration in plasma ultrafiltrate in mEq. per kg. water. Sodium space may be calculated similarly. In normal muscle, sodium space is slightly larger than chloride space, indicating that some sodium may be intracellular or 'bound' to the cell surface (cf. Manery, 1954). In skin, where some chloride is known to be bound to extracellular collagen, the chloride space is larger than the sodium space. Sodium and chloride spaces in rat skin and muscle are shown in Table II. Once the size of the extracellular fluid volume is established, the

TABLE II

DERIVED DATA: RAT MUSCLE AND SKIN

Same data as in Table I. Methods of calculation are shown in the text

	$(H_2O)_t$	$(H_2O)_e^{Cl}$	$(H_2O)_e^{Na}$	$(H_2O)_i$	$[Na_i+K_i]$
	g./100 g. FFDT				mEq./kg. H_2O
Muscle	339	50	63	289	138
Skin	210	166	136	73	81

intracellular fluid volume may be calculated by subtraction of the extracellular fluid volume from total tissue water. I shall assume that in muscle

$$(H_2O)_i = (H_2O)_t - (H_2O)_e^{Cl} \qquad (3)$$

If one assumes with Conway (1957) that some chloride is intracellular, and that $[K]_e = [Cl]_i$, then chloride space as calculated from Equation 2 overestimates the extracellular fluid volume by about 10 per cent. In injured tissues it is much less certain that chloride is strictly extracellular. To my knowledge, there have been no attempts to compare chloride space with inulin space or sucrose space in injured mammalian muscle, so I shall attempt to interpret

the water and cation changes on the basis of the simultaneous chloride changes.

One may determine the difference in water content between injured and control muscle, and compare this with the difference in chloride space between the two muscles. If the increment in total water exceeds the increment in chloride space then this water must be in a space not occupied by chloride. The extra water is presumably in the intracellular phase:

$$\Delta\ (H_2O) > \Delta(H_2O)^{Cl}_e \quad \textit{Increased intracellular water.}$$

On the other hand, if the increment in chloride space exceeds the increment in total water, then chloride must have invaded a space it did not previously occupy:

$$\Delta(H_2O)^{Cl}_e > \Delta(H_2O) \quad \textit{Invasion of intracellular space by chloride.}$$

Of course if the total water and the chloride space increase equally, then the intracellular fluid volume can be assumed to have remained unchanged.

The concept that cells are iso-osmolar with extracellular fluid has been questioned recently (cf. Robinson, 1953). However, the most careful determinations of freezing point of muscle compared with that of plasma indicate equality of osmotic pressure in the two phases (cf. Conway, 1957). If the concentration of intracellular solutes is calculated from tissue analyses and $(H_2O)_I$, the intracellular osmolarity is only slightly greater than the extracellular osmolarity. If allowance is made for bound intracellular ions the intracellular and extracellular osmolarities are about equal. It is therefore not necessary to postulate the existence of a 'water pump' continuously expelling water from the cell in order to maintain an intracellular hyper-osmolarity. The simplest hypothesis is that the cells and extracellular fluid are in osmotic equilibrium and that water passes freely across the membranes separating them.

The maintenance of an intracellular fluid high in potassium and low in sodium may be most satisfactorily explained by an hypothesis based upon the electrochemical gradient across the cell membrane and an active extrusion of sodium from the cell. When muscle is at rest sodium remains in the extracellular phase, not because it cannot penetrate the membrane, but because the sodium which enters is

continuously extruded by an active metabolic process – the 'sodium pump'. Recent evidence suggests that in several tissues extrusion of sodium is linked to the entrance of potassium (cf. Hodgkin, 1958), but in either case outward movement of sodium occurs against an electrochemical gradient. Both potassium and chloride readily pass across the membrane of the resting muscle cell. The electrical potential difference between the outside and inside of the muscle cell is fairly close to that predicted from the potassium concentrations in the two phases:

$$E = \frac{RT}{F} \ln \frac{[K]_i}{[K]_e} = 58 \log_{10} \frac{[K]_i}{[K]_e} \qquad (4)$$

in which E is the Potential Difference, R is the Gas Constant, T is the Absolute Temperature and F is the Faraday. Some comparisons between the membrane potential measured across muscle-cell membranes and the potassium equilibrium potential calculated from Equation 4 have been tabulated recently by Shanes (1958). In skeletal muscle and ventricular muscle the differences are usually less than 10 mV. Modification of Equation 4 by introduction of terms for a sodium 'leak' would make the agreement even better (cf. Hodgkin, 1958).

The nature of the 'sodium pump' is still unknown. The immediate source of energy for the active sodium transport is probably ATP or related compounds (Fuhrman, 1952; Fleckenstein, Gerlach and Janke, 1956). It is probable that, depending upon the tissue, either glycolysis or respiration may furnish energy for sodium transport.

EFFECT OF INJURY ON TISSUE ELECTROLYTES

If plasma accumulated at sites of injury, there would be no change in the concentration of electrolytes in circulating plasma. However, it is well known that animals and men have hyperkalaemia and hyponatraemia after injury, and Manery and Solandt (1943) demonstrated a marked loss of potassium and gain in chloride in traumatized muscle. Thus the injured tissues are at least partly the cause of the abnormalities of the circulating plasma.

In 1945 Tabor and Rosenthal, and independently Fox and Keston, showed that whole legs of mice, following tourniquet injury, contained more sodium than could be accounted for by the increased

fluid present if it was assumed that this fluid contained sodium at the concentration present in plasma ultrafiltrate. Since coincident loss of tissue potassium occurred, it was presumed that the sodium entered the intracellular phase. This 'excess sodium', i.e. sodium not present in the extracellular water, has since been demonstrated following several different types of injury. Some of these are listed in Table III. Data from our own experiments on muscle are plotted in Fig. 1. The straight line is drawn to represent the increase in muscle sodium which would result from addition of simple extracellular fluid. The points above the line show the presence of 'excess sodium'. This figure also includes examples of mild injuries in

TABLE III

INJURED TISSUES IN WHICH 'EXCESS SODIUM' HAS BEEN FOUND

Injury	*Tissue*	*Reference*
Tourniquet	Mouse leg	Tabor and Rosenthal, 1945 Fox and Keston, 1945
Scald	Mouse leg	Fox and Baer, 1947
Frostbite	Rabbit muscle	Crismon and Fuhrman, 1946
Tourniquet	Rat muscle	Fuhrman and Crismon, 1951*b* Ravin *et al.*, 1954 MacPhee, 1955
Tourniquet	Rabbit muscle	Fuhrman and Crismon, 1951*a*
Pentothal; CN′	Rabbit muscle	Fuhrman, 1951*a*
Microsphere Ischaemia	Rabbit muscle	Fuhrman *et al.*, 1951
Flash Burn	Rat skin	Walser and Bodenlos, 1954*b*

which 'excess sodium' was not found. Although there is no doubt about the existence of 'excess sodium' after a variety of severe injuries, this simple fact does not show what other alterations in electrolyte patterns have been produced by injury.

Rather than describe the electrolyte changes in various types of injury, I shall attempt to classify the electrolyte patterns found in injured tissues in general. Four representative patterns in muscle are shown in Fig. 2. I have selected animals from our own experiments which show distinct patterns. The four types of electrolyte pattern shown are (A) the pattern in normal muscle, (B) the pattern of simple oedema, (C) cation shift with normal or large intracellular volume and (D) cation shift with small intracellular volume.

Normal (A). The figures are means of determinations on gastrocnemius muscles from seventeen rabbits. The sodium space exceeds

the chloride space by about 5 g./100 g. FFDT. This is equivalent to less than 1 mEq./100 g. FFDT intracellular sodium.

Simple oedema (B). Total tissue water is greater than in normal muscle and the increment in water content is the result of increase in extracellular fluid volume only. Chloride space and sodium space

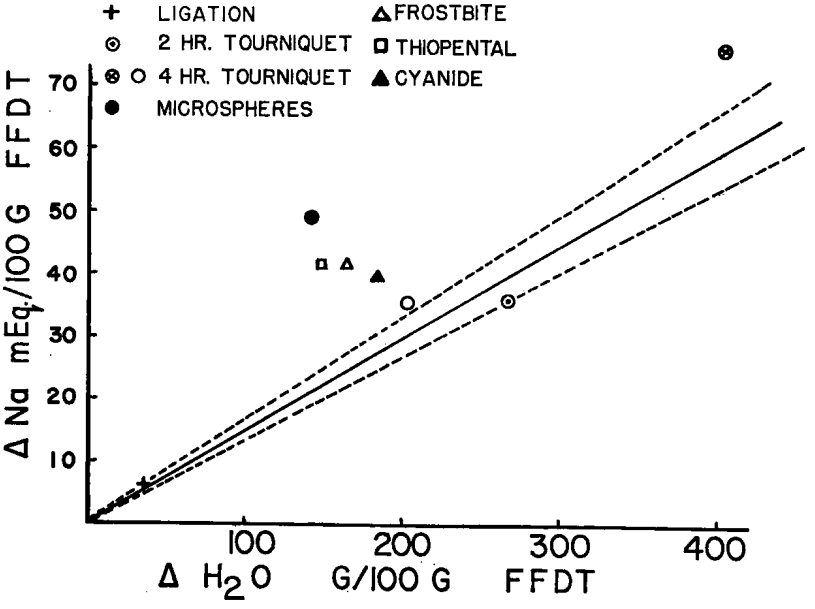

FIG. 1. 'EXCESS SODIUM' IN INJURED SKELETAL MUSCLE

The difference in amount of sodium (mEq. per 100 g. FFDT) between injured and uninjured muscle is plotted as a function of the difference in amount of water (g. per 100 g. FFDT) between the two muscles. The straight line is drawn to represent the increase in muscle sodium which would result from addition of simple extracellular fluid. The interval between the dotted lines indicates the range of sodium concentrations lying between the limits of ±1 standard deviation. The points above the line show the presence of 'excess sodium'.

are both enlarged by the same amount as is total water. In a perfect example of simple oedema there would be no decrease in tissue potassium per unit dry weight. I presume that others at this conference will discuss simple oedema in detail. We have found that it occurs only rarely in experimental animals after injury. The example shown is gastrocnemius muscle from a rabbit one day after the intra-arterial injection of thiopental into a limb occluded by a tourniquet around the thigh (Fuhrman, 1951*a*). We have observed simple