Cellular Nucleotides in Hemorrhagic Shock: *

Relationship of Intestinal Metabolic Changes to Hemorrhagic Enteritis and the Barrier Function of Intestinal Mucosa

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"Je dis à dessein 'autopsie physiologique', parce qu'il n'y a que celles-la qui soient réellement instructive. C'est la disparition des propriétés physiologiques qui expliques la mort, et non pas les altérations anatomiques." ††

A SEARCH for the critical factors which govern survival after shock led us first to a study of oxygen consumption during hemorrhagic shock and after re-infusion. Guyton and Crowell provided the lead with the demonstration that recovery bore a relationship to the depression of over-all oxygen consumption during the hypotensive period.[18] To this we were able to add the observation that in animals which had reached the irreversible stage the total oxygen consumption did not recover to pre-shock levels following re-infusion.[46] The next step was to identify the organ which had thus become incapable of normal oxygen utilization despite the resumption of an adequate flow of oxygenated blood. It was found that as hemorrhagic hypotension progressed beyond a certain point in time, the resumption of an adequate mesenteric blood flow after re-infusion became ineffective in raising oxygen consumption in the intestine of the dog, although the liver and limbs resumed normal blood flow and an almost normal uptake of oxygen.[5]

The previous finding of an over-all depression of oxygen consumption after re-infusion in irreversibly shocked animals was confirmed, and to this information was added the demonstration of a specific profound depression in the oxygen uptake of the intestine.

Thus it became of compelling interest to determine the significance, if any, of the apparent metabolic breakdown within the intestine. Did this additional demonstration of concomitance between irreversibility and a defect in oxygen consumption, now shown to occur in the intestine before any other tissue studied, play a determinant role in the progressive deterioration leading to the death of the organism as a whole?

The question arose also as to whether blood was being shunted away from the mucosal cells or whether an actual altera-

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†† Bernard, C., Introduction à l'étude de la médecine experimental, Paris, Librairie Ch. Delagrave, 1903, p. 251.

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tion in cellular metabolism had occurred in the intestine.

It was this latter question which led us originally to an *in vivo* study of the rate of turnover of phosphate (P_{32}) in the nucleotides and inorganic phosphate present in the acid soluble fraction of the intestine and liver.[6] On the basis of this study it was concluded that the change in mesenteric oxygen consumption associated with irreversible shock did indeed reflect severe impairment in both the biosynthesis of adenosin triphosphate and the rate of oxidative phosphorylation within the mucosal cells of the intestine.

Despite the evident importance of this discovery, the answers to certain questions became even more urgent. Did alterations in local hemodynamics precede or follow the metabolic deterioration? Where did the constant finding of hemorrhagic enteritis fit into the hemodynamic and metabolic picture? Accepting the importance of a breakdown in biosynthetic and energy-producing activity in the intestine, what relationship could it bear to the rapid demise of the animal within a period of two to 12 hours? The rapidity of the fatal collapse suggested an overwhelming toxic episode, and led us to speculate upon the possibility that the intestinal mucosa, damaged in its intrinsic mechanisms upon which its own defense depends, might be abnormally vulnerable to assault by the normal content of the animals own intestine.

This paper deals with the role of the intestine in death from hemorrhagic shock. We shall attempt to identify the critical events at the cellular level, to trace their genesis through the altered biology of the tissue involved, the intestinal epithelium. The interpretation which follows is tentative, rough, incomplete, but would seem unified.

Experimental Method

Healthy young dogs averaging 14 Kg. were used; anesthesia was obtained using intravenous pentobarbital with intubation to maintain an adequate airway. The shock preparation consisted in rapid bleeding of the animal through a femoral arterial catheter into a reservoir bottle until a level of 30 mg. Hg mean arterial pressure was reached. This pressure was maintained for 30 minutes, after which the level of the bottle was raised to provide a pressure of about 50 mm. Hg, this latter pressure being maintained for a selected period of time. The blood in the reservoir was then reinfused over a period of 10 or 15 minutes.

The duration of the period of hypovolemia, and accordingly the decision to retransfuse in irreversibly shocked dogs (Group III and IV), was based upon the appearance of significant signs of impending irreversibility such as loss of reflexes in a lightly anesthetized dog, rather than on a strict time schedule. Mesenteric blood flow was measured by a direct technic described in a previous communication.[5] A brief outline follows. P_{32} in a dose of 10/μc/Kg. (sodium phosphate at pH 6) was injected into the afferent half of the extracorporeal apparatus used to measure blood flow. In the unshocked controls the injection was made after a 10 to 15 minute stabilization period, whereas in the shocked groups reported here the P_{32} was injected about 10 minutes after the re-infusion of shed blood. Intestinal tissue samples were taken from the terminal ileum one, two, three, five, ten, 15 and 30 minutes following the injection of P_{32}, and the liver samples after 10 and 30 minutes.

The samples of tissue excised were washed at once in ice cold saline and placed upon a cold Petri dish. The epithelial lining was then scraped carefully from the muscularis mucosae; histologic examination showed that the mucosal specimen consisted only of columnar epithelium. The mucosal and muscular portions of the specimen were placed in two separate centrifuge tubes containing 2 ml. of 10 per cent trichloracetic acid. It was thus possible to

compare simultaneously the rate of incorporation of P_{32} into the various fractions of the metabolically active mucosa and the less active muscular layer, the latter serving in each sample as a control for the mucosal specimen.

The determination of specific activity of the nucleotide phosphate (mainly adenosine triphosphate) and inorganic phosphate present in the acid soluble fractions of the tissue homogenates was made by the method described previously.[6] The nucleotide phosphates were divided into two fractions. The first fraction contained the two outer phosphates of the ATP molecule (pyrophosphates) and reflected the rate of oxidative phosphorylation. The second fraction contained the innermost phosphate (total nucleotide phosphate) and reflected the rate of synthesis of the nucleotide molecule.

Four experimental groups comprise the present report. Groups one, two and three furnished data some of which have been reported elsewhere.[6] However, all three of these groups provide the essential controls for the new data provided by Group four. The need to reinterpret the data reflecting the hemodynamic state in each group, and the requirements of lucidity have obliged us to present the results of all four groups together.

Group I. Control group, not shocked (five dogs).

Group II. Sublethally shocked survivors in which re-infusion of blood was started after 90 minutes of hypovolemia (four dogs).

Group III. Irreversibly shocked dogs in which blood was re-infused after 120 to 150 minutes of hypovolemia (five dogs).

Group IV. (Six dogs.) The shock procedure and method of study was exactly the same as for Group III, all animals being irreversibly shocked after a hypovolemic period of 120 to 170 minutes. In this group, however, ileal loops were studied in the following manner. At the beginning of the experiment, before bleeding was commenced, a 50 to 60 cm. segment of lower ileum was occluded between Pott's vascular clamps applied so as to compress only the intestinal wall without interference with the mesenteric blood supply, either arterial or venous. Into this isolated segment (loop "a") was then injected 75,000 K.I.U. of a proteinase inactivator commercially known as Trasylol,* diluted in 40 ml. of saline. Another clamp was placed on the bowel about 15 to 20 cm. proximally, thus isolating a second segment of ileum (loop "b"). The content of this second loop was washed out by lavage with about 4 L. of warm saline at moderate pressure; 25,000 K.I.U. of Trasylol in 10 ml. of saline were left inside the loop. The intestinal samples following P_{32} injection after reinfusion were taken from loop "a" at the usual time intervals. An additional sample was taken from the second intestinal segment "b" 30 minutes after the injection of P_{32} in order to compare the mucosal and muscular metabolic activity with that of the first loop not washed of its intestinal content. It was thus possible to study whether or not the presence of the intraluminal contents of the bowel exerted any detectable influence upon the course of the metabolic changes in the intestinal mucosa during shock.

Results

In Figures 1 and 2 and Tables 1–3 are illustrated the average specific activities for the inorganic phosphates and nucleotide phosphates over the time course following injection of radiophosphorus. Labeled inorganic phosphorus is carried into the capillaries by flow, where it diffuses out of these vessels and becomes distributed in the interstitial and intracellular spaces accessible to phosphate. The height of the peak was found to be about five times as great as that for the tissue; the specific activity of the blood phosphate decays rapidly to a steady state level of the order of approximately two times the specific activity present in the tissue (Fig. 8). At this time the specific activity observed in the tissue *in toto* reflects the intracapillary, extra-capillary and intracellular specific activity. The initial rate of rise and the first peak are consequent to the distribution of isotope into the capillary bed by flow, and its diffusion into the tissues. The fall is con-

* Schultz, F. Uber ein biologisch Hoakaktives Polypeptid, Den Kallikrein inaktivator (Trasylol). Med. und Chem. V11/750/1963.

sequent to removal of the label from the capillary bed by flow, and the diffusion back into the capillary bed of extravascular label. Recirculation of isotope results in a steady state value which declines slowly. The time of appearance of the peak of specific activity following intravenous injection of P_{32} in a given tissue of the body is related to the velocity of the systemic circulation and to the cardiac output, while the difference observed between two adjacent tissues in the same organ conceivably reflects the peculiar pattern of the local capillary density and resistance.

The height of the peak is interpreted as reflecting the volume of circulating blood in the given tissue at the time of excision. Whatever the hemodynamic interpretation, a typical pattern is constantly seen in the normal dog: the peak in radioactivity is reached in the mucosal tissue in half the time noted in the corresponding muscular coats with a slightly elevated peak in actual radioactivity (Fig. 1–3). The filling time of the mucosal vascular network, in comparison with the muscular layer, reflects a greater velocity of circulating blood with lower peripheral resistance. The lower steady level of radioactivity is also reached in half the time in the mucosal tissue. In Group II (Fig. 1, 4, Table 1) reversibly shocked dogs, the ratio of blood velocity

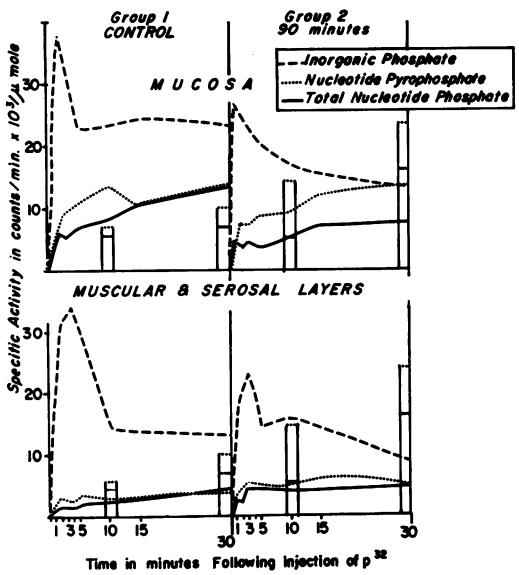

FIG. 1. Average specific activity in count/m/μ mole of inorganic phosphate, nucleotide pyrophosphate and total nucleotide phosphate in ileum mucosa (upper half), muscular and serosal layers of ileum (lower half) and liver (column).

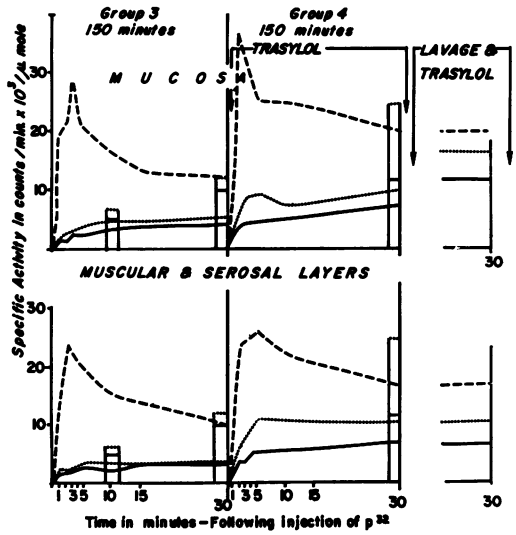

FIG. 2. Keys as in Fig. 1.

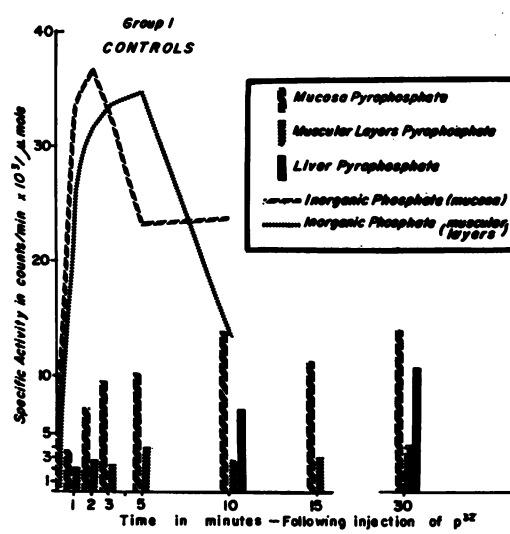

FIG. 3. Average specific activity in counts/m/μ mole of inorganic phosphate and nucleotide pyrophosphates in mucosa, muscular and serosal layers of ileum and in the liver.

TABLE 1. *Specific Activity of Non-nucleotide Inorganic Phosphate*

Time (min.) After Injection of P32		1	2	3	5	10	15	30
Mucosa	Group I	33,970±8,510[5]	36,560±5,990[5]	33,440±5,070[5]	22,930±3,830[5]	34,750±3,900[4]	24,380±3,720[5]	23,260±4,800[5]
	Group II	27,000±4,386[4]	26,000±4,360[4]	23,224±4,028[4]	20,740±3,970[4]	17,264±1,414[4]	15,284±2,588[4]	12,970±1,760[4]
	Group III	20,280±3,330[5]	20,600±2,310[5]	28,550±3,970[5]	22,500±2,370[5]	16,440±2,270[5]	13,400±1,250[5]	12,350±1,730[5]
	Group IVa	24,598±3,549[6]	35,608±4,500[6]	30,158±3,131[6]	24,918±1,120[6]	24,650± 970[6]		19,584±2,045[6]
	Group IVb							19,352±1,630[6]
Muscular	Group I	25,430±5,590[5]	32,790±5,450[5]	33,580±8,980[5]	34,690±7,010[5]	13,520±2,040[5]	12,270±1,410[5]	12,840±2,390[5]
	Group II	18,460±3,268[4]	15,950±1,576[4]	23,684±2,884[4]	14,040±2,108[4]	17,000±1,250[4]	12,230±1,226[4]	9,224±1,102[4]
	Group III	13,120±2,110[5]	16,540±2,000[5]	24,570±3,950[5]	21,110±2,820[5]	15,730±3,000[5]	14,510±4,450[4]	10,550±2,240[5]
	Group IVa	14,728±2,481[6]	23,574±2,719[6]	22,940±2,714[6]	25,200±2,192[6]	21,798±2,147[6]		16,016±1,343[6]
	Group IVb							16,490±1,159[6]
Liver	Group I					14,190± 580[2]		16,580± 800[2]
	Group II					15,180±5,886[4]		17,440±4,282[4]
	Group III					12,050±1,410[5]		16,730±2,420[4]

All values are shown as mean±S.E.M. specific activity in counts/m/μ mole.
No. animals in group shown by superscripts.
Group I. Control.
Group II. Dogs that survived 90 minutes of hypovolemia.
Group III. Dogs that did not survive 150 min. of hypovolemia.
Group IV. Dogs that did not survive 150 min. of hypovolemia. Loop "a" was pretreated with Trasylol. Loop "b" was pretreated with lavage+Trasylol.
Note: The superscripts refer only to tables 2 and 3.

TABLE 2. *Specific Activity of Nucleotide Pyrophosphates*

Time (min.) After Injection of P32		1	2	3	5	10	15	30
Mucosa	Group I	3,440± 880[5]	7,280±2,180[5]	9,450±1,800[5]	10,120±2,760[5]	13,980±3,810[5]	11,160±1,900[5]	14,010±2,540[5]
	Group II	4,602±1,060[4]	7,200± 962[4]	7,114±1,070[4]	8,134±1,896[4]	9,310±1,476[4]	12,000±2,600[4]	13,400±3,114[4]
	Group III	1,920± 460[5]	1,710± 260[5]	2,570± 250[5]	3,690± 410[5]	4,330± 640[5]	4,530± 670[5]	5,730± 640[5]
	Group IVa	2,572± 802[5]	5,314± 747[5]	8,724±1,658[5]	9,082±1,609[5]	7,270±1,042[5]		10,040±1,011[5]
	Group IVb							16,212±1,655[5]
Muscular	Group I	2,180± 380[5]	2,600± 580[5]	2,370± 570[5]	3,760± 700[5]	2,330± 250[5]	2,920± 530[5]	3,950± 680[5]
	Group II	3,532± 333[4]	4,226± 694[4]	5,304±1,388[4]	4,634± 264[4]	5,750±1,458[4]	7,450±1,510[4]	5,960± 846[4]
	Group III	2,300± 30[5]	1,930± 510[5]	2,060± 270[5]	3,850± 790[5]	3,940± 430[5]	3,960± 660[5]	4,210± 640[5]
	Group IVa	3,044± 846[5]	5,288± 694[5]	6,944± 859[5]	11,026±2,561[5]	10.050±1,919[5]		10,522±1,391[5]
	Group IVb							10,374±1,377[5]
	Group I					7,060±1,080[2]		10,320±1,450[2]
	Group II					14,804±3,524[4]		24,124±4,504[4]
	Group III					6,620±1,600[5]		12,560±2,030[4]
	Group IV							24,304±6,378[5]

TABLE 3. *Specific Activity of Total Nucleotide Phosphates*

Time (min.) After Injection of P32		1	2	3	5	10	15	30
Mucosa	Group I	3,800±1,250[5]	6,210±1,610[5]	5,630±1,180[5]	6,900±1,430[5]	8,180±1,790[5]	10,550±1,750[5]	13,080±2,160[5]
	Group II	4,576± 872[4]	4,034± 884[4]	4,454±1,176[4]	4,304± 992[4]	5,434±1,474[4]	7,730±1,908[4]	8,000±1,474[4]
	Group III	1,700± 550[5]	1,500± 290[5]	2,110± 170[5]	2,700± 290[5]	3,330± 370[5]	3,350± 430[5]	4,140± 510[5]
	Group IVa	2,058± 380[6]	3,932± 418[6]	4,126± 562[6]	4,446± 497[6]	5,280± 777[6]		7,342±1,491[6]
	Group IVb							11,500±1,905[6]
Muscular	Group I	1,480± 520[5]	2,020± 640[5]	1,180± 380[5]	2,290± 500[5]	2,350± 390[5]	2,920± 470[5]	4,660±1,580[5]
	Group II	2,952± 602[4]	2,604± 308[4]	4,126±1,212[4]	4,234± 654[4]	4,000± 934[4]	1,912± 722[4]	4,934± 948[4]
	Group III	1,830± 200[5]	1,890± 240[5]	1,820± 250[4]	2,690± 540[5]	2,080± 320[5]	3,110± 390[5]	3,430± 550[5]
	Group IVa	2,274± 312[6]	3,844± 459[6]	3,814± 459[6]	5,180± 988[6]	5,926±1,557[6]		6,996±1,279[6]
	Group IVb							6,254±1,077[6]
Liver	Group I					5,540± 70[2]		7,000± 960[2]
	Group II					3,200±1,118[4]		16,022± 125[4]
	Group III					5,000± 960[5]		9,880±1,250[4]
	Group IV							11,644±1,971[6]

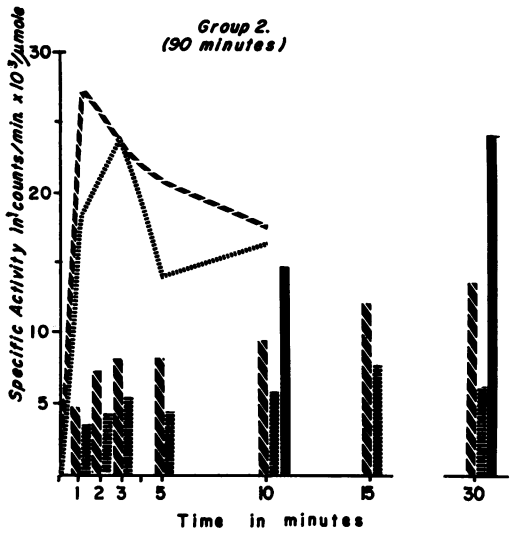

FIG. 4. Key as in Fig. 3.

and volume of the mucosa tissue versus muscular layer is the same as controls. The peak occurs after one minute in the mucosa and after two minutes in the muscular layer; the pattern of tissue microcirculation does not appear altered so far as the reciprocal characteristics of the two tissues are concerned. The systemic pattern, however, is different because the speed with which the radioactive material reaches the periphery is markedly increased as is the cardiac output in reversibly shocked animals following the retransfusion of blood.

In these dogs (Group II) however, a major biochemical change is noticeable in the mucosa. A marked depression is noted in the rate of labeling of the innermost phosphate in the mucosal nucleotides (Fig. 1, Table 3). The specific activity of the total nucleotide phosphate in the control group was constantly about three times higher than the corresponding values in the muscular layers throughout the 30 minutes sampling period ($P < 0.01$), in Group II it is only 0–60 per cent above the value for the muscular layer 30 minutes after injection of P_{32} ($P < 0.50$). Similarly, when compared to the corresponding value in the liver, the S.A. for total nucleotides in the mucosa is found to be depressed to about half the corresponding value for the liver while it was above it in the control. Considering the specific activity of the pyrophosphate fraction in the mucosa in comparison with muscle and liver in Group II (Fig. 3, 4) it should be noted that the turnover of this fraction is markedly increased in the muscular layers as it is in the liver, reflecting the marked increase of total body O_2 consumption which was observed previously in survivors.[5] Nevertheless, the rate of turnover of the pyrophosphate in the mucosal nucleotides is still from 100 to 130 per cent above the corresponding value for the muscular layers ($P < 0.05$), it is also only about half the value for the liver while it was above it in controls. It is thus apparent that the mucosa does not participate, in survivors, in the over-all increase in oxidative phosphorylation observed in other tissues following retransfusion of blood.

These changes occur in the mucosa when the O_2 delivery through the capillary network is reestablished to normal as shown by the inorganic phosphate curves (Fig. 4). It is reasonable to assume that, since these

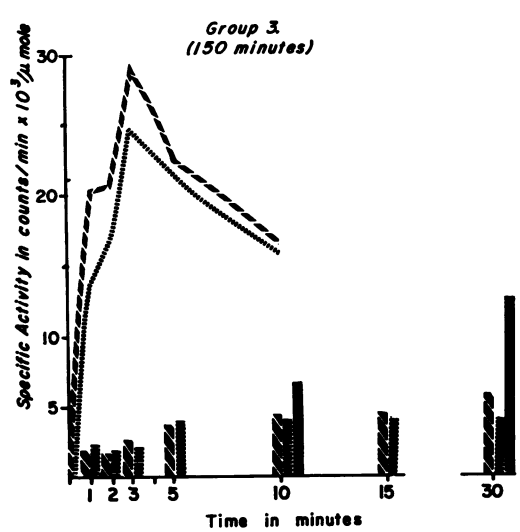

FIG. 5. Key as in Fig. 3.

dogs are survivors, these metabolic changes are temporary and a return to normal will occur in time and before irreversible changes have taken place in the cells.

The trend observed in sublethal cases is accentuated in Group III (Fig. 2). Mucosa and muscular layers now show the same rate or synthesis of the nucleotides. In this group the turnover of P_{32} in the nucleotide pyrophosphates in the mucosa is depressed to the value of the muscular layers reflecting an impairment in the oxidative phosphorylation to about one-third of control (Fig. 5, Table 2). Fifteen minutes after injection of P_{32} the specific activity of the nucleotide pyrophosphate in the mucosa of the control group (Group I) is 11,160 and in Group III it is 4,530 ($P < 0.01$). Thirty minutes after injection of the isotope the corresponding values are 14,010 and 5,730 ($P < 0.01$) with no significant changes in the muscular layers. A new feature is also present in Group III regarding the specific activity of inorganic phosphate. The curve depicting this fraction shows a definitely delayed appearance of the peak in specific activity which now occurs simultaneously with that of the muscular layers (3 min. after injection). No significant difference is seen in the actual height of radioactivity. In these five dogs only slight hemorrhagic lesions were visible

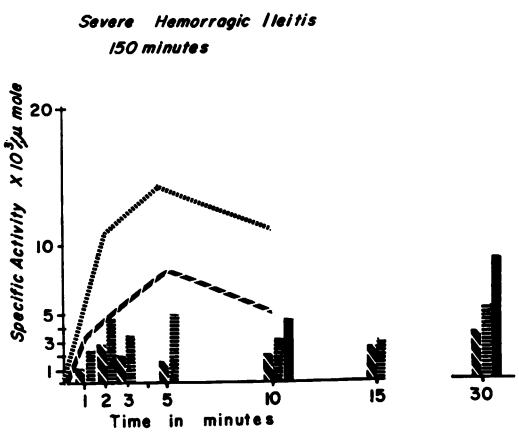

FIG. 6. Key as in Fig. 3.

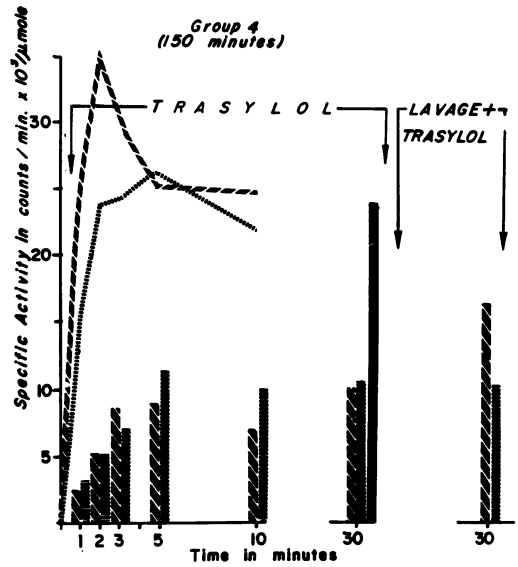

FIG. 7. Key as in Fig. 3.

in some mucosal samples; two individual dogs in which marked hemorrhagic infarcts were observed in the mucosa at the time of excision are separately illustrated in Figure 6. These last two animals showed not only a delayed appearance, but also an

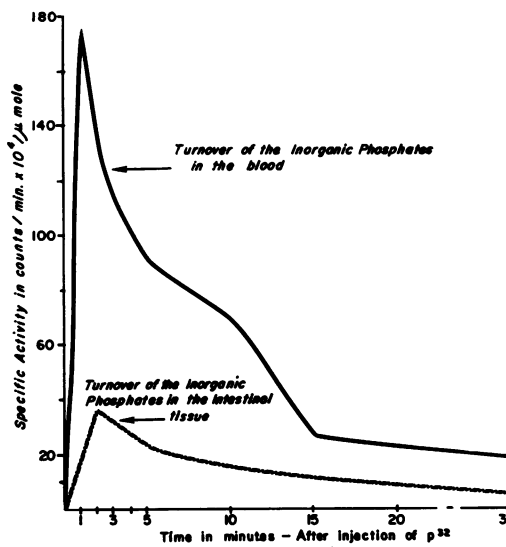

FIG. 8. Curves relating to the specific activity of the inorganic phosphate in the blood and in the intestinal tissue following injection of radiophosphorus.

over-all depression in the height of specific activity for inorganic phosphates in the mucosal tissue when compared to the muscular layers.

The interpretation of these findings in terms of capillary hemodynamics is, at present, a matter of speculation. The phosphate is carried along the capillary and diffuses extravascularly. If the capillaries are quite permeable, then it would be expected from the formulation of Goresky [15] that the rate of propagation of the electrolyte would be delayed by a factor related to the size of the accessible extravascular space, that is, if tissue becomes edematous, or hemorrhage occurs into the tissue, the phosphate concentration wave would travel more slowly along the capillaries. This factor would explain the delay observed in the peak of specific activity in the mucosal tissue. The depression in total radioactivity observed in the mucosa of some dogs (Fig. 6) is attributed to a reduction in the accessible capillary bed.

Group III animals showed the typical hemorrhagic ileitis so frequently observed in association with irreversible shock in the dog. The intestines of animals of Group IV also showed this lesion but the loops isolated by clamps and treated with Trasylol with or without lavage showed little or no evidence of mucosal hemorrhage.

The turnover of inorganic phosphate in the mucosa of the Trasylol treated loop in Group IV (Fig. 2, 7) shows a pattern almost precisely similar to control dogs. These animals show no visible lesions in the excised mucosa. The peak of specific activity of inorganic phosphate in the mucosa is reached two minutes after injection and in the muscular coat after five minutes; the height of radioactivity is also considerably higher in the mucosa than in the muscular coat. The elevation of inorganic phosphate level in the mucosa of these dogs demonstrates that the size of the accessible capillary bed is increased above normal following retransfusion, indicating a state of reactive hyperemia. In comparison with untreated bowel in irreversibly shocked (Group III) dogs, the hemodynamic conditions are excellent in the mucosa of these dogs following retransfusion of blood, as is the macro- and microscopic morphology.

The turnover of the pyrophosphate fraction is, however, markedly depressed in the mucosal cells: 10 minutes after injection of the label, the specific activity in the mucosa is 7,270 and 30 minutes after is 10,040 while the corresponding value for the muscular coats are 10,050 and 10,522, respectively, a drop in mucosal oxidative phosphorylation of even greater magnitude than in Group III. Similarly the total nucleotide phosphate fraction in the mucosa is reduced to approximately the level of the muscular layers. Furthermore, the specific activity of the liver's pyrophosphate fraction is more than twice the mucosal value (24,304) as in Group III. By contrast, the second loop of intestine, from which intestinal content was removed by lavage prior to injection of Trasylol, shows a significant difference in metabolic activity. The mucosal pyrophosphate in the sample of intestine excised from this loop shows a specific activity of 16,212 compared with 10,374 in the corresponding muscular layers. Thirty minutes after injection of P_{32} no difference is seen between the muscular layers of the two samples from the washed and unwashed loop. However, a significant difference is noted between the two mucosal fractions: each individual experiment shows a greater oxidative phosphorylation of the mucosa of the washed side than with the nonwashed loop. The study of the total nucleotide fraction confirms a greater metabolic activity on the washed side. The specific activity of this fraction is 11,500 in the mucosa and 6,254 in the muscular layers of the washed loop

($P < 0.05$); the corresponding values for the first or unwashed loop 30 minutes after P_{32} injection are 7,342 and 6,996.

Comment

Pathogenesis of Hypovolemic Hemorrhagic Enteritis. A survey of the extensive literature of past years reveals that the only reliable findings by which one may distinguish between an early (reversible) and a late (irreversible) stage of shock in the dog is a pathologic change: hemorrhagic necrosis of the intestinal mucosa. It is understandable then that a great deal of effort has been devoted to the study of this change. The observation, in dogs subjected to prolonged hemorrhagic shock, of intense intestinal and hepatic congestion with a 10 to 15 mm. Hg increase in portal pressure has led most investigators to implicate an obstruction to the venous outflow of the liver with damming of blood in the portal system causing engorgement, congestion, and hemorrhage in the intestine.[14, 30, 39, 49, 52] The quest for an anatomical basis for this theory has led several authors to describe a sphincter mechanism either functional or structural in the venous outflow system of the canine liver.[3, 11, 25, 27, 45, 47] "The much discussed but infrequently demonstrated sphincter mechanism in the hepatic veins" [29] is still the subject of much controversy. Guntheroth [16] has pointed out that many of the *physiologic* studies upon which the existence of these sphincters is based were largely pharmacologic studies, often with extirpated organs, using indirect methods and under unnatural conditions. Indeed, the anatomic existence of these sphincters has been recently challenged,[41] while their role in producing the characteristic pathology in the intestine of the dog in shock as opposed to humans is certainly diminished by Elias's demonstration [12] of comparable amounts of smooth muscle in the hepatic veins of man. Miyake [28] has observed that the hepatic veins in humans are equipped with a thick muscular coat while the portal branches have very little muscle, quite comparable to the situation which exists in dogs. It would thus appear anatomically possible for the human to react to shock in the same manner as described for the dog. Furthermore, a sizable group of hemodynamic studies, in dogs, have not supported the concept of a damming of blood in the portal system in relation to the irreversibility of shock.[22, 33, 50] Our previous studies [5] are in keeping with the findings of other workers [2, 10, 35, 36, 43] who demonstrated a reactive hyperemia of the splanchnic area with increased hepatic flow following re-infusion of blood.

As we approached the problem of the haemorrhagic intestinal lesion in the dog, a certain number of observations made us dissatisfied with the classical hemodynamic interpretation of these lesions:

Congestion is not synonymous with hemorrhage in several congestive human clinical conditions. In particular clinical portal hypertension of far greater magnitude than observed in the retransfused dog does not necessarily produce intestinal capillary hemorrhage.

In our studies during hypovolemia we have observed that the hemorrhagic lesions are already present before blood is retransfused at a time when portal pressure was found to be below normal values (average 8 mm. Hg): the explosive effects of internal hydrostatic pressure could not be intelligently implicated under these circumstances.

The anatomical distribution of the hemorrhage over the mucosal surface is patchy and rather segmental for several hours; only on the following day would the lesions be homogenous, suggestive of a causative mechanism of equal intensity diffused over the entire intestinal surface, as would be expected if hepatic resistance was of prime importance. Furthermore, there is a lack of analagous lesions in other splanchnic areas drained by tributaries of the portal venous system.

Our previous studies [5] of mesenteric circulation with the portal vein divided and bypass of the hepatic resistance revealed that, following retransfusion of blood, these dogs showed a percentage increase of mesenteric blood flow in relation to cardiac output similar to those animals in

which the mesenteric blood flow was studied with the portal vein and thus hepatic resistance intact. Before reaching the level necessary to rupture the capillary wall the hydrostatic intraluminal pressure should be expected to produce a change in the pressure head gradient sufficient to affect the volume of flow in the portal vein. The concomitant observation of a high oxygen content in the portal vein following reinfusion [5] was further evidence to support the fact that portal hypertension is a consequence of increased blood flow produced by a reactive hyperemia in the splanchnic area.

We found evidence of intestinal hemorrhage in some dogs with the portal vein divided, in keeping with Lillehei's observation [26] that these changes still occur even in animals where the portal system was decompressed by the prior construction of an Eck fistula.

Finally, it was the simple observation of the time course of events under the microscope that brought us to the solution of this *splanchnic enigma.* It was our preconceived belief that the purpose of explaining a phenomenon is better achieved by observing how it originates rather than how it ends: indeed, the much publicized submucosal hemorrhages seen by the pathologist on the following morning when the dog had been dead for some hours was confirmed by us. Earlier specimens excised at the first appearance of the hemorrhage revealed that these findings are but the terminal event of a process which actually originated in the interstitial tissue of the villi in the area immediately facing the intestinal lumen. As the time and severity of the lesion progressed, the vascular lesion extended deeper in the mucosa towards the muscular layer, finally involving the submucosal spaces (Fig. 9–12). Because of the greater caliber of the vessels in the submucosa and the lack of unimpeded access of the blood to the empty spaces inside intestinal lumen, the blood accumulates more easily in the submucosal space. The suspicion arose in our minds that the unknown factor might be found inside the intestinal lumen. By washing a segment of the ileum with saline we were able to prevent the occurrence of the massive hemorrhagic lesion (Fig. 13), and so confirm this assumption. The next step was, of course, to attempt to identify the particular substance responsible for these lesions. The well-known role of trypsin in the production of experimental hemorrhagic pancreatitis suggested to us that perhaps a similar phenomenon could occur under conditions of shock in the intestinal mucosa. Indeed, the mechanism that protects the epithelial cell of the ileal mucosa from proteolytic digestion by enteric proteases has puzzled investigators since the time of Claude Bernard. Although the exact mechanism is unknown it is conceivably based upon energy-production through oxidative phosphorylation. Since our studies [5] have shown that the oxygen consumption of the intestine is greatly reduced during hypovolemia despite the increased arteriovenous difference, it was logical to expect a depression in this protective mechanism of the mucosal cells. This hypothesis was confirmed when two different specific trypsin inhibitors (amino caproic acid and Trasylol—Fig. 14, 15) definitely protected the intestinal mucosa from hemorrhage. It was also found that it is possible to produce *ad libitum* hemorrhagic lesions in any chosen segment of the mucosa, in mild hemorrhagic shock, by the local application of activated trypsin (Fig. 16). The independence of *congestive* phenomena from the *hemorrhagic* is also shown in the microscopic picture of the Trasylol-treated loops of irreversibly shocked dogs following reinfusion of blood; epithelial cells are normal, there is no extravasation of blood, but evidence of dilated venules and reactive hyperemia is present.

Hemodynamic and Metabolic Phenomena in the Mucosa of the Intestine During Shock. We had thus succeeded in dissociating the hemodynamic and metabolic phenomena in the intestine in shock. Having elucidated the pathogenesis of the hemorrhagic lesions, it became of great interest for us to establish whether the hemodynamic changes observed in the mucosa in the dogs of Group III and reflected in the delayed appearance of the peak in inorganic phosphate radioactivity and also by a severe depression in the total amount of radioactivity in the dogs of Figure 6, were related to the vascular hemorrhagic phenomena and, if so, what role did they play in relation to the simultaneously observed metabolic depression in the mucosal tissue. The opportunity was now available to us; we had developed an experimental preparation in which one of the two phenomena was abolished. The turnover of P_{32} into the various phosphorus containing fractions was repeated in Group IV in exactly the same way as in Group III, except that the tissue samples were excised from a loop of ileum previously treated with Trasylol and in which hemorrhagic lesions did not develop. It was found that both oxidative

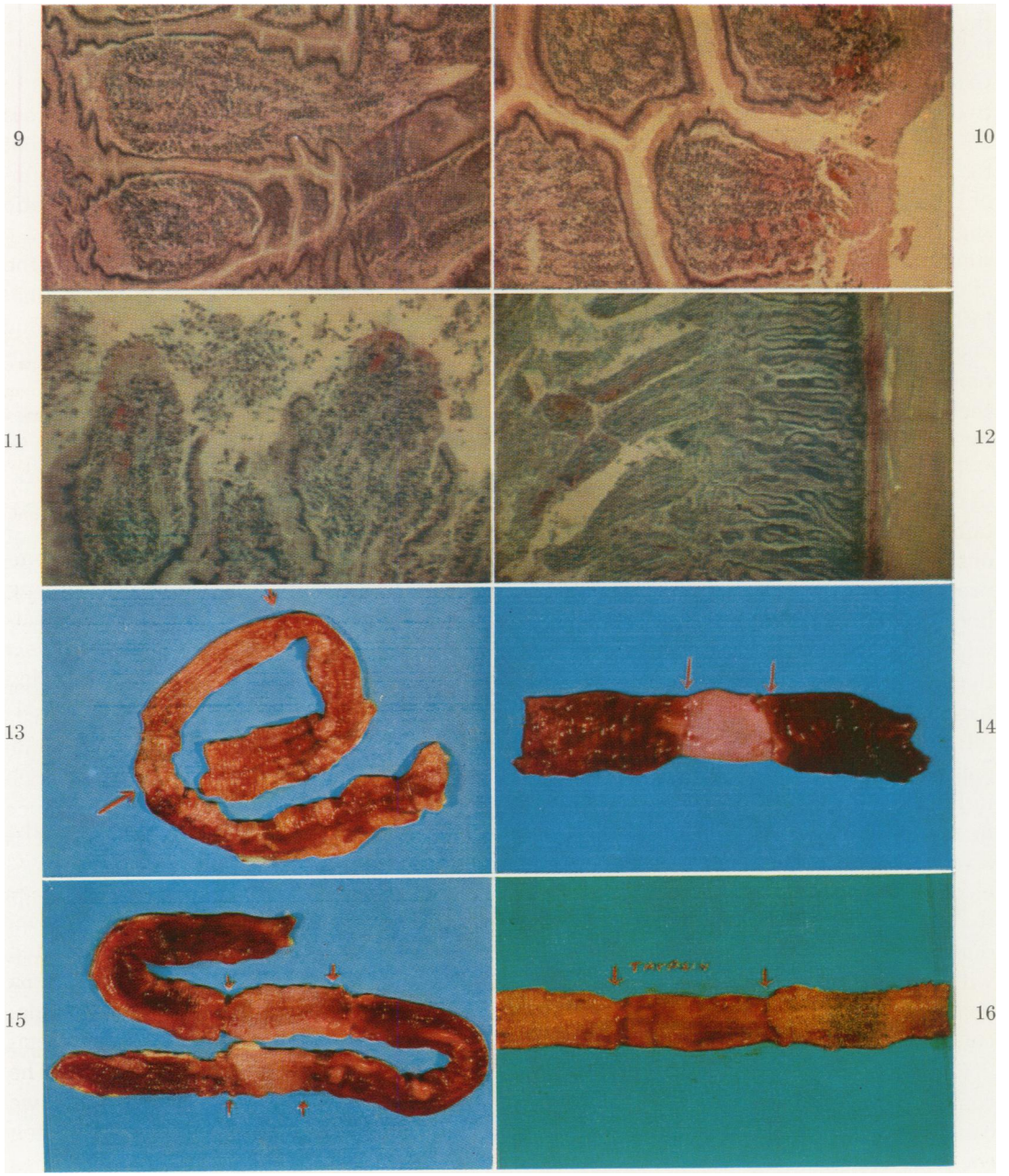

FIG. 9–12. These four sections show the progressive penetration of the hemorrhagic lesions beginning at the tips of the villi and working toward the submucosa. The fragmentation of the luminal portion of the villi is pathological in nature, as the untouched surface of the mucosa has been fixed by the direct injection of the Bouin fixative into the lumen of the bowel without prior washing. The luminal side of the section is on the left in Figures 9 and 12, on the right in Figure 10, and on the top in Figure 11. The stain is hematox-eosin. FIG. 13. The effect of lavage of an intestinal segment before shock on the development of hypovolemic hemorrhagic lesions. FIG. 14. The effect of local treatment with trypsin inhibitors (area between arrows) on the development of hemorrhagic lesion in the ileum. FIG. 15. Two separated segments of the ileum have been pretreated: the upper one with lavage and trasylol, the lower one only with trasylol: both segments are equally protected from the development of hypovolemic hemorrhagic ileitis. FIG. 16. The effect of local application of trypsin (area between arrows) in a reversible hemorrhagic shock preparation.—Histology by D. Hodges, Photography by H. Artinian.

phosphorylation and nucleotide synthesis in the mucosa were depressed to or below the level of the muscular layer as observed in Group III; the significant difference between the two groups was the finding of an absolutely normal curve of specific activity for the inorganic phosphate in the mucosa of the Trasylol-treated loop. The hypothesis suggested itself that the reduced O_2 consumption observed in hypovolemia, and also following retransfusion of blood in the intestinal mucosa of irreversibly shocked dogs, impairs the energy-dependent defense mechanisms which protect the epithelial cells of the mucosa from dissolution by intraluminal proteases.

The fact that the normal tissue hemodynamic pattern can be artificially maintained in the mucosa of shocked dogs without favorably affecting the metabolic depression justifies our conclusion. The metabolic depression is the primary phenomenon which favors the development of the hemodynamic changes through digestion of the parenchymatous and vascular structure by intraluminal trypsin. This finding invalidates the theory of a selective vasospasm in the mucosal tissue as a pathogenic factor in mucosal hemorrhagic necrosis. The delayed transport of the blood-flow dependent electrolyte appears to be a consequence of the altered microcirculation of blood produced by the disruption of the vascular tree with hemorrhage and thrombosis. The prevention of the latter does not change the course of the metabolic phenomena.

Perhaps a factor which has tended to complicate the actual detection of the causative event is that each phenomenon could independently cause the other. The alteration in the normal hemodynamic pattern, as reflected by delayed turnover of the inorganic phosphates, whether it be capillary rupture or constriction, could produce through ischemic-anoxia the observed metabolic depression, while the depression in oxidative metabolism could bring about the hemodynamic changes through trypsin-dependent capillary digestion. The possible role of trypsin itself upon coagulation or sludging has not been the subject of the present investigation. Numerous studies [13, 37, 38] have shown that proteolytic enzymes affect the coagulation of blood in different ways and could conceivably play an important role in secondary local hematic phenomena.

If, then, selective vasospasm is not the primary cause, why should the intestinal mucosa be affected prior to any other tissue by an equivalent reduction in oxygen supply?

Hypothesis Concerning the Pathogenesis of the Metabolic Changes Observed in the Intestinal Mucosa. The higher respiratory and mitotic rate of the intestinal mucosa did not appear sufficient to account for its greater sensitivity to ischemia; indeed, nature has provided the highly respiring tissues with a greater capillary density so that any reduction in flow of blood would be proportional. On the other hand, tumors do not seem to resent particularly the effect of ischemia. A unique feature of the intestinal epithelium which occurred to us as of potential significance was the intimate contact with feces. It is well known that numerous powerful poisons are normally found in the feces. The role of the mucosa in forming a first line of defense against the entry of noxious materials is little understood.[42] Some of these poisons such as histamine [24] and other products of bacterial origin would have a devastating effect on the systemic arterial vasculature were they to pass unaltered through the intestinal barrier.[1, 42] Indeed, histamine has been found to increase in the circulating blood during endotoxin shock.[20] In this paper, however, we would like to draw attention to another group of poisonous substances normally found in the intestines, substances having a well documented direct histotoxic effect on cellular respiration. Some are of bacterial origin like the cyanide produced by

pseudomonas aeruginosa,[9] or bacterial endotoxin.[7] Other substances found in considerable amount in the feces are indole and skatole.[19, 48] These substances, if injected intravenously in the dog in sufficient amounts, produce a fall in blood pressure, convulsive movements and death by cardiac dilatation. Perhaps of greater importance might by the marked effect of indole upon cellular respiration and phosphate transport.[23, 24]

It is known that during the advanced hypovolemic phase of severe shock the functions of most organs are depressed, for example, protein production by the liver, the visual function of the retina, the contractile strength of skeletal muscle, the secretory function of the gastro-intestinal tract. Similarly it has been shown that intestinal absorption and transport of glucose and amino acid is reduced.[17] Similarly the absorption of water is markedly depressed.[34] Let us now consider a second function of the intestinal mucosa that is indeed unique, the little understood barrier function. Even a momentary depression of this function could allow the penetration inside the mucosal cells of the histotoxic substances previously described, whether of bacterial or strictly chemical nature. It is conceivable that a certain period of time must elapse during which these powerful poisons are intracellular before they can ultimately reach the opposite end of the cell and eventually the blood stream. The reduction in cellular oxidative phosphorylation and blood flow in all tissues of the body, produced by the decreased cardiac output, could be perpetuated, following retransfusion of blood, in the epithelial lining of the intestine by intracellular penetration of substances capable of inhibiting oxydative phosphorylation and related metabolic processes. Indeed, this hypothesis appears to be justified by our observations. Both oxidative phosphorylation ($P < 0.05$) and nucleotide synthesis ($P < 0.05$) are significantly higher in the mucosa previously washed as compared with the adjacent mucosa in which only the prevention of cellular autolysis has been provided for through the local application of trypsin inhibitor. With regard to the rapid cellular dissolution produced by trypsin, it should be kept in mind that, as a faster process masks a slower one, the details and implications of the predigestive phase of the cellular metabolic breakdown could hardly be studied without adequate protection from cellular dissolution.

Relevance of the Described Shock Studies to Clinical Shock Problems. Moore [29] in summarizing the present knowledge on shock said "the most important irrelevance of laboratory study in understanding human shock lies in circulatory rather than cellular matters and especially in the difference of hepatosplanchnic circulatory arrangements as between the dog and man . . . by contrast the death of the cell involves biochemical changes that are common to all vertebrate species." We have now demonstrated that the intestinal lesions specific for the dog are the result of the action of trypsin upon a metabolically depressed intestinal mucosa; they can be prevented with trypsin inhibitor or enhanced by activated trypsin. The differences in trypsin content of succus entericus could conceivably explain differences in occurrence and intensity of intestinal hemorrhage. To quote again from Moore's article regarding the difference between dogs and humans we read further of the dog that "its dietary habit consists of infrequent meals eaten after seizing, fighting and killing its prey, followed by digestion of the entire victim including skin, hair, and skeleton." It is conceivable indeed that such an animal might be equipped by nature with a more efficient and altogether different rate of digestive enzyme production. The literature pertaining to this subject is scarce indeed. Some relevant facts can be found supporting the concept that a difference does exist between dogs, rats and mice on the one

hand, and humans on the other. Zucker [51] found continuous secretion in his pancreatic fistula dogs. Thomas [44] in a survey of the literature, concluded that human pancreatic juice consistently contained less protein than that of dogs. Little is known about the fate of pancreatic enzymes after they enter the intestine.

Cajori [8] observed in dogs a wide variation in enzyme activity in different samples of intestinal juice from the same loop without correlation of the difference in enzyme activity with the food intake of the animal: proteolytic activity was present in every sample. Pelot [32] studied the trypsin activity in the rat's intestine and found that all samples contained readily measurable amounts of trypsin. In humans, on the other hand, Shwachman [40] using a very sensitive method, observed that in the groups above 10 years of age, stool trypsin was practically absent in all specimens: the only cases with demonstrable trypsin in the stool were patients suffering from gastroenteritis. Borgstrom [4] noted that in humans the pancreatic secretion started 10 to 20 minutes after ingestion of a test meal and then continued to flow as long as there was food in the stomach. In regard to this discontinuity of pancreatic secretion it should be kept in mind that pancreatic enzymes undergo rapid inactivation on entering the small intestine as proven by the fact that with pancreatic fistula these enzymes disappear within a few hours from the intestinal content.[44] A species difference in pancreatic excretion would certainly affect the enteric proteolytic activity. As discussed above it appears that tryptic digestion and consequent hemorrhage in the intestinal mucosa represent the final event that follows a metabolic cellular change. While the former is species-dependent, the latter of a more specific biochemical nature could be common to both humans and dogs. The morphology of the cell throughout the period of biochemical breakdown as seen by conventional histology was found to be absolutely normal until the abrupt destruction of the architecture of the cell, presumably due to proteolytic activity.

We consider the interval of time between the onset of functional deterioration and the breakdown of anatomical integrity to be an area of challenge for future investigation. The order of disappearance of life-sustaining metabolic processes must lie hidden in the intestinal mucosa in the period preceding cellular disintegration.

Summary and Conclusions

A technic employing P_{32} to label *in vivo* the nucleotides of intestine and liver has been used to study the visceral damage which follows the ischemic anoxia of hemorrhagic shock in the dog. Samples of intestine and liver taken after P_{32} injection, during a control period and following reinfusion of blood, have shown a profound depression of oxidative phosphorylation and of nucleotide synthesis in the intestinal mucosa in irreversible shock. The time curves of the specific activity of inorganic phosphorus in the intestine, together with gross and microscopic observations, have provided a sequential study of hemodynamic alterations and of the development of hemorrhagic enteritis. The following summarizes the principal observations:

The metabolic deterioration in the mucosa of the intestine appears to reach an irreversible stage *before* the appearance of detrimental alterations in the hemodynamics of the intestine.

The metabolic depression renders the mucosal cells permeable by intraluminal proteolytic enzymes such as trypsin. The characteristic hemorrhagic enteritis of late shock in the dog is produced by this mechanism.

Hemorrhagic enteritis is prevented by inactivating intraluminal tryptic ferments by means of a protease inhibitor, Trasylol.

The inactivation of trypsin in the presence of intraluminal stool has no favorable effect upon the development of the metabolic depression in the mucosa. However, when Trasylol application is combined with lavage of the bowel to remove stool, the metabolic deterioration is delayed.

The advent of the severe metabolic depression in the intestinal mucosa is conditioned not only by low blood flow for a critical time interval, but also by the direct contact with intestinal content other than trypsin.

The intestinal mucosa in late shock undergoes an alteration in its normal function as a barrier. The breakdown of the barrier is not dependent upon the prior development of hemorrhagic enteritis. Therefore, the serious consequences of a loss of barrier function might quite conceivably develop in species in which the trypsin-induced hemorrhagic enteritis is not ordinarily a feature of shock, such as in man.

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