

Guest Editorial

THE OCCURRENCE of negative nitrogen balance is one of the central problems of inflammatory states whether the cause is trauma (including impact, burns, radiation, heat, or cold injury), infection, allergy, or nonallergic reactions to drugs or chemicals. Dr. Cuthbertson was the first to report the development of negative nitrogen balance in trauma (1) and has reviewed the problem in detail (2). Elsewhere in this issue (3) Drs. Cuthbertson and Tilstone review this aspect of trauma and neatly categorize and assess the importance of the immediate reaction to injury and the onset of catabolism. They discuss the secondary factors of decreased food intake and immobilization and the role of the environmental temperature.

The biochemical mechanisms leading to negative nitrogen balance in injury must ultimately be explored in order for the problem to be solved. Thus, it might be useful to inquire whether our present knowledge of protein biosynthesis and degradation can be analyzed in such a way that a biochemical approach can be made to the problem of the negative nitrogen balance in injury. Obviously, the direct study of biochemical changes in injured patients is most difficult if not impossible. Extensive physiological studies are often impractical because of the circumstances of the injury. However, the biochemical changes can be studied in traumatized animals to determine what specifically should be looked for in the injured man.

Extensive studies have been carried out to determine the mechanism of protein synthesis and degradation. In addition, a large number of metabolic pathways exist for transforming amino acids into other nonprotein substances and ultimately into

urea. The mechanism of protein synthesis is presently believed to be a complicated process. The information as to what the amino acid sequence of a protein should be is transmitted from deoxyribonucleic acid (DNA) by means of a messenger ribonucleic acid (mRNA) to an assembly point on a particulate body, the ribosome. Amino acids, having been transported in an aminoacyl form attached to a transport ribonucleic acid (tRNA), are arranged in sequence on the ribosome. The entire process involves a great number of distinct pathways. (See, for example, references 4-8.)

- 1) Biosynthesis of purine and pyrimidine ribo- and deoxyribonucleoside triphosphates.
- 2) Biosynthesis of DNA from the appropriate deoxyribonucleoside triphosphates.
- 3) Biosynthesis of mRNA, tRNA, and rRNA (ribosomal ribonucleic acid) at specific areas of DNA (the gene) in order that the proper sequence of purine and pyrimidine bases is obtained.
- 4) Biosynthesis of the unusual purine and pyrimidine bases found in tRNA (9).
- 5) Formation of aminoacyl tRNA (10).
- 6) Biosynthesis of ribosomes from rRNA and ribosomal protein (11, 12).
- 7) Assembly of protein, protein precursors, and protein subunits from aminoacyl tRNA utilizing ribosomes and mRNA and several other cofactors (7, 13, 14).
- 8) Formation of the final protein by transformation of the protein precursor or assembly of protein subunits (15, 16).

- 9) Degradation of DNA and RNA by specific deoxyribonucleases and ribonucleases (17).
- 10) Degradation of protein into peptides and amino acids by various proteolytic enzymes including intracellular enzymes (cathepsins).
- 11) Conversion of amino acids into urea.

In addition to these reactions, other reactions must be considered from the control point of view.

- 12) Induction of protein synthesis by substrate and repression by product at the DNA level (18-22).
- 13) Feedback and other non-DNA regulatory controls within each specific pathway.
- 14) Hormonal regulation of each of the specific pathways.

There are also several other reactions whose physiological importance is not yet certain.

- 15) Biosynthesis of histones (23-29).
- 16) Methylation of tRNA (30).
- 17) Action of endonucleases and the nucleic acid repair process (17, 31).
- 18) Formation of polyribosomes (6).

Finally, one must consider other amino acid pathways.

- 19) Nonprotein transformations of amino acids.
- 20) Transport of amino acids into and out of cells and cell compartments.

Some of these pathways are difficult to study in *in vitro* preparations. However, it is possible to look at other of these reactions in animals subjected to trauma. It would seem to be necessary now to study as many of these reactions as possible *in vitro* in a specific animal subjected to a standardized traumatic procedure at specific time intervals in several tissues. Thus, a systematic approach would give information as to the time course of change, if any, of some of the pathways involved in

protein synthesis and degradation in several tissues after trauma. Rather than expecting that a specific pathway might be drastically altered, one might find that very specific proteins are synthesized at a decreased rate whereas others are synthesized at an increased rate. If this were the case, then the induction and repression of specific protein synthesis would have to be invoked. This at least would account for the differential response of plasma proteins in injury (e.g., decrease in plasma albumin, increase in plasma fibrinogen) (32). Tissue damage might release materials that have specific inductive and repressive effects on protein synthesis.

The number of pathways involved in protein synthesis is large and the study of each is quite complex. It is possible that different pathways in different tissues are affected by injury. Conversely, injury of different tissues may affect different pathways.

It is notable that in the injured state an increase in oxygen consumption and heat production occurs. This suggests that there is an increase in exothermic reactions or an uncoupling of oxidative phosphorylation in mitochondria, or both. It would be of interest to examine mitochondrial preparations obtained from acutely injured animals and to measure the oxygen uptake and ATP production. If changes in mitochondrial function can be demonstrated, even if they are secondary, this might give a clue as to the locus of some of the intracellular changes that occur in injury. If injury releases potent substances from tissue it might be possible to detect these substances in the serum of injured patients by their effect on mitochondrial function *in vitro*.

Since injury leads to the disruption of the normal steady-state metabolism of the organism there must be a signal mechanism whereby tissue damage influences not only the intermediary metabolism of damaged and adjacent tissue but also that of more distant organs. The kinin sys-

tem could theoretically fulfill this role. This involves the following reactions (33-39).

- 21) Hageman factor is transformed into activated Hageman factor (blood clotting factor XII).
- 22) Activated Hageman factor activates plasma thromboplastin antecedent (PTA, blood clotting factor XI).
- 23) Activated Hageman factor also transforms proactivator into activator for plasminogen.
- 24) Plasminogen is transformed into plasmin by activator.
- 25) Activated Hageman factor may lead to the formation of permeability factor.
- 26) Permeability factor or activated Hageman factor, or both, transforms kallikreinogen into kallikrein.
- 27) Either, or both, plasmin and kallikrein act on an alpha-2 globulin (kininogen) to produce kallidin-10, a decapeptide.
- 28) An aminopeptidase splits off lysine forming kallidin-9, or bradykinin.

Bradykinin increases the blood flow of sweat glands, salivary glands, and exocrine glands, is a powerful vasodilator, stimulates and relaxes visceral smooth muscle, dilates capillaries, increases the permeability of the capillary endothelium, causes pain, and stimulates white blood cell migration. Undoubtedly, the kinin system is more complex than indicated here and many more details remain to be uncovered. For example, it has recently been reported that there is a kallikrein inhibitor that forms an inactive complex with kallikrein. This kallikrein inhibitor may be identical with the C'1-esterase inhibitor of the complement system of the blood that is missing in hereditary angioneurotic edema (40-43). Perhaps bradykinin, or some similar substance, or the depletion of this kallikrein inhibitor, or

both, leads to an increase in cell membrane permeability. An increase in cell permeability leads to the intracellular accumulation of sodium and water (intracellular edema) and the loss of intracellular potassium. Similar alterations involving mitochondria may lead to the uncoupling of oxidative phosphorylation with increased oxygen uptake and heat production with decreased ATP formation. In isolated mitochondria depleted of potassium the uptake of oxygen is decreased and then increased by the addition of potassium (44). Valinomycin, which causes an increased uptake of potassium by mitochondria, causes a further increase in oxygen uptake (44-46). It has been suggested that mitochondria might be able to take up potassium from the extracellular fluid even when the cell itself is unable to accumulate intracellular potassium (44). Other uncoupling agents of mitochondria also enhance potassium uptake (47). Perhaps the increased oxygen uptake and heat production seen in injury represent potassium accumulation in mitochondria secondary to an earlier state of potassium depletion. In the earlier depleted state decreased oxygen uptake and heat production might occur with decreased protein synthesis (with no change, or perhaps an increase in protein degradation). The loss of nitrogen and the increase in oxygen uptake and heat production may be only the later manifestations of the earlier primary event. In this regard it is most important to document the exact time course of oxygen uptake, heat production, nitrogen balance, and potassium loss in the injured state. As Drs. Cuthbertson and Tilstone indicate urinary potassium rises early after injury whereas the loss of nitrogen and increased heat production are later events. It might be supposed that the potassium loss occurs from the site of injury, but quantitation is necessary before this can be stated with certainty. It would be important to calculate how much potassium is lost from an in-

jured site in an animal and how much from other sites.

Because protein synthesis involves a complex series of reactions the negative nitrogen balance in injury may be expected logically to be due to many different changes. Although this discussion has been speculative, the analysis of possible metabolic mechanisms occurring in injury at least brings out some of the considerations one must take into account in approaching this problem. Despite the complexity of the problem a beginning has to be made if ever we are to understand the nature of the changes that occur in injury.

ROBERT H. HERMAN, M.D.

Metabolic Division

*U. S. Army Medical Research
and Nutrition Laboratory
Fitzsimons General Hospital
Denver, Colorado*

REFERENCES

- CUTHBERTSON, D. P. The disturbance of metabolism produced by bony and non-bony injury with notes on certain abnormal conditions of bone. *Biochem. J.* 24: 1244, 1930.
- CUTHBERTSON, D. P. Physical injury and its effect on protein metabolism. In: *Mammalian Protein Metabolism*, edited by H. N. Munro and J. B. Allison. New York: Academic, vol. II, 1964, p.373-414.
- CUTHBERTSON, D. P., AND W. J. TILSTONE. Nutrition of the injured. *Am. J. Clin. Nutr.* 21: 911, 1968.
- KORNER, A. Protein biosynthesis in mammalian tissues. I. The mechanism of protein synthesis. In: *Mammalian Protein Metabolism*, edited by H. N. Munro and J. B. Allison. New York: Academic, vol. I, 1964, p. 177-242.
- HELINSKI, D. R., AND C. YANOFSKY. Genetic control of protein structure. In: *The Proteins. Composition, Structure, and Function* (2nd. ed.), edited by H. Neurath. New York: Academic, vol. IV, 1966, p. 1-93.
- STENT, G. S. Coupled regulation of bacterial RNA and protein synthesis. In: *Organizational Biosynthesis*, edited by H. J. Vogel, J. O. Lampen and V. Bryson. New York: Academic, 1967, p. 99.
- LIPMANN, F., Y. NISHIZUKA, J. GORDON, J. LUCAS-LENARD AND M. GOTTESMAN. Bacterial amino acid polymerization. In: *Organizational Biosynthesis*, edited by H. J. Vogel, J. O. Lampen and V. Bryson. New York: Academic, 1967, p. 131.
- VOGEL, H. J., AND R. H. VOGEL. Regulation of protein synthesis. *Ann. Rev. Biochem.* 36: Part II, 519, 1967.
- FITTLER, F., L. K. KLINE AND R. H. HALL. Biosynthesis of N⁶-(Δ^2 -isopentenyl) adenosine. The precursor relationship of acetate and mevalonate to the Δ^2 -isopentenyl group of the transfer ribonucleic acid microorganisms. *Biochemistry* 7: 940, 1968.
- NOVELLI, G. D. Amino acid activation for protein synthesis. *Ann. Rev. Biochem.* 36: Part II, 449, 1967.
- STAEHELIN, T., H. RASKAS AND M. MESELSON. Taking the ribosome apart and putting it back together again. In: *Organizational Biosynthesis*, edited by H. J. Vogel, J. O. Lampen and V. Bryson. New York: Academic, 1967, p. 443.
- NOMURA, M., AND P. TRAUB. Structure and function of ribosomes and subribosomal particles. In: *Organizational Biosynthesis*, edited by H. J. Vogel, J. O. Lampen and V. Bryson. New York: Academic, 1967, p. 459.
- HAENNI, A-L., J. LUCAS-LENARD AND J. GORDON. Function of the T-GTP-aminoacyl-sRNA complex in polypeptide formation. *Federation Proc.* 27: 397, 1968.
- SUTTER, R. P., AND K. MOLDAVE. The interaction of aminoacyl transferase II and ribosomes. *J. Biol. Chem.* 241: 1698, 1966.
- CLARK, J., AND D. STEINER. The biosynthesis of insulin in the rat. *Federation Proc.* 27: 393, 1968.
- SJOERDSMA, A., S. UDENFRIEND, H. KEISER AND E. C. LEROY. Hydroxyproline and collagen metabolism. *Ann. Internal Med.* 63: 672, 1965.
- LEHMAN, I. R. Deoxyribonucleases: Their relationship to deoxyribonucleic acid synthesis. *Ann. Rev. Biochem.* 36: Part II, 645, 1967.
- PARDEE, A. B., F. JACOB AND J. MONOD. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β -galactosidase by *E. coli*. *J. Mol. Biol.* 1: 165, 1959.
- JACOB, F., AND J. MONOD. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* 3: 318, 1961.
- BECKWITH, J. R. Regulation of the lac operon. *Science* 156: 597, 1967.
- MAGASANIK, B. Catabolite repression. *Cold Spring Harbor Symp. Quart. Biol.* 26: 249, 1961.
- GOLDBERGER, R. F., AND M. A. BERBERICH. De-repression and repression of the histine operon: Sequential and simultaneous modes. In: *Organizational Biosynthesis*, edited by H. J. Vogel, J. O. Lampen and V. Bryson. New York: Academic, 1967, p. 199.
- SHIMONO, H., AND A. S. KAPLAN. Histone synthesis

- in cells infected with a virulent or an oncogenic virus. *Federation Proc.* 27: 615, 1968.
24. ALLFREY, V. G., V. C. LITTAU AND A. E. MIRSKY. On the role of histones in regulating ribonucleic acid synthesis in the cell nucleus. *Proc. Natl. Acad. Sci., U. S.* 49: 414, 1963.
 25. ALLFREY, V. G., R. FAULKNER AND A. E. MIRSKY. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc. Natl. Acad. Sci., U. S.* 51: 786, 1964.
 26. SONNENBERG, B. P., AND G. ZUBAY. Nucleohistone as a primer for RNA synthesis. *Proc. Natl. Acad. Sci., U. S.* 54: 415, 1965.
 27. CHALKLEY, G. R., AND H. R. MAURER. Turnover of template-bound histone. *Proc. Natl. Acad. Sci., U. S.* 54: 498, 1965.
 28. COMMERFORD, S. L., AND N. DELIHAS. Examination of the nucleohistone from mouse liver and intestine for RNA covalently linked to histone. *Proc. Natl. Acad. Sci., U. S.* 56: 1759, 1966.
 29. BONNER, J., AND J. WIDHOLM. Molecular complementarity between nuclear DNA and organ-specific chromosomal RNA. *Proc. Natl. Acad. Sci., U. S.* 57: 1379, 1967.
 30. FENRYCH, W., W. FALERYCH, H. HUANG AND B. C. JOHNSON. Methylation of *E. coli* t-RNA. *Federation Proc.* 27: 795, 1968.
 31. OLIVERA, B. Enzymatic joining of DNA. *Federation Proc.* 27: 395, 1968.
 32. OWEN, J. A. Effect of injury on plasma proteins. In: *Advances in Clinical Chemistry*, edited by H. Sobotka and C. P. Stewart. New York: Academic, vol. 9, 1967, p. 1-41.
 33. ALDRETE, J. S., S. G. SHEPS, P. E. BERNATZ AND E. P. DIDIER. Vasoactive polypeptides. *Mayo Clin. Proc.* 41: 399, 1966.
 34. WEBSTER, M. E. The kallikrein-kininogen-kinin system. *Arthritis Rheumat.* 9: 473, 1966.
 35. COLMAN, R. W., AND S. SHERRY. Isolation, purification and physicochemical properties of the plasma kallikreins. Abstracts, 60th Annual Meeting, Soc. Clin. Invest., Atlantic City, N. J., May 6, 1968, p. 22a.
 36. MITCHELL, J. C. Bradykinin (a vasoactive polypeptide). *New Engl. J. Med.* 271: 1057, 1964.
 37. LEWIS, G. P. Plasma kinins and inflammation. *Metab. Clin. Exptl.* 13: 1256, 1964.
 38. RATNOFF, O. D. Increased vascular permeability induced by human plasmin. *J. Exptl. Med.* 122: 905, 1965.
 39. LEWIS, G. P. Pharmacological actions of bradykinin and its role in physiological and pathological reactions. *Ann. N. Y. Acad. Sci.* 104: 236, 1963.
 40. SHERRY, S., AND R. COLMAN. Observations on the plasma kallikreinogen-kallikrein enzyme system. 81st Annual Meeting, Assoc. Am. Phys., Atlantic City, N. J., May 7, 1968.
 41. DONALDSON, V. H. Serum inhibitor of C1-esterase in health and disease. *J. Lab. Clin. Med.* 68: 369, 1966.
 42. YACHNIN, S. Functions and mechanism of action of complement. *New Engl. J. Med.* 274: 140, 1966.
 43. AUSTEN, K. F. Inborn errors of the complement system of man. *New Engl. J. Med.* 276: 1363, 1967.
 44. GORDON, E. E., K. NORDENBRAND AND L. ERNSTER. Evidence for a new mechanism of respiratory stimulation and proton ejection in Ehrlich ascites tumor cells dependent on potassium ions. *Nature* 213: 82, 1967.
 45. COCKRELL, R. S., E. J. HARRIS AND B. C. PRESSMAN. Energetics of potassium transport in mitochondria induced by valinomycin. *Biochemistry* 5: 2326, 1966.
 46. HARRIS, E. J., R. COCKRELL AND B. C. PRESSMAN. Induced and spontaneous movements of potassium ions into mitochondria. *Biochem. J.* 99: 200, 1966.
 47. LYNN, W. S., AND R. H. BROWN. Effects of uncoupling agents on respiration, cation exchange, and phosphorylative efficiency in mitochondria. *Arch. Biochem. Biophys.* 114: 271, 1966.