Volume 24, Number 5
September–October 1963

THE JOURNAL OF Anesthesiology
THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, INC.

Editorial Views

Protein Binding of Anesthetic Molecules

In a recent article in *Pharmacological Reviews,* Featherstone and Muehlbaecher gently attempted to divert, at least partially, the attention of anesthetists and pharmacologists from fifty or more years of preoccupation with lipoid molecules by directing their thoughts to the roles proteins might play in anesthesia produced by some of the small non-hydrogen bonding molecules, such as xenon, ethylene and cyclopropane. The suggestion was made that protein molecules of various kinds, a few now being well defined as chemical entities, but most still known only by a more general set of characteristics, play roles not only in the absorption, distribution, possible metabolism and the excretion of anesthetics, but also in the actual mechanisms by which these substances produce anesthesia.

An article on competitive protein binding and potentiation of pentobarbital anesthesia by Lasser, Elizondo-Martel and Granke in this issue of Anesthesiology supports the idea that proteins in biological systems are as important in the various facets of anesthesia with barbiturates as they may be with the small gaseous or volatile anesthetic molecules. The article by Lasser and his colleagues does not present a new type of observation, since it describes another example of the phenomenon of potentiation of barbiturate sleeping time. However, the speculations concerning protein binding are of considerable theoretical interest. The potentiating agent described is a compound called Urokon, which alone causes central nervous system stimulation. It is a contrast substance used by radiologists, and is a different type chemically than other compounds known to potentiate barbiturate sleeping time.

The data presented in this paper only indirectly implicate albumin as the object of competition between Urokon and pentobarbital, but pertinent *in vitro* experiments from the literature are cited to support the hypothesis that Urokon binds to albumin firmly and allows a higher level of free or unbound pentobarbital to be presented for entrance into the central nervous system. Compounds like Urokon are known to be bound to proteins so firmly that protein-bound iodine studies are not reliable for as long as a year after Urokon-type compounds have been given.

However, now that roles for proteins are being recognized and described with greater frequency, there is some danger that "proteins" will join "permeability," "altered metabolic pathway" and other catch phrases which have hidden hosts of researchers for many years. If success is to be achieved in understanding events, now defined to a considerable extent in physiologic terms, in the language of the underlying fundamentals of chemistry and physics, more researchers must be engaged in studying to greater depths interesting phenomena of the type reported by Lasser *et al.*, so that the end result of such research efforts need not be that "protein binding must have occurred."

One must ask how the binding occurred, with which protein(s) and with what conse-

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quences. Brodie, Gillette, Shanker, and their colleagues have published a number of papers in recent years which would suggest extensions of the Lasser *et al.* research in several directions. The data presented do not allow one to know whether the pentobarbital metabolizing enzyme systems in the liver are affected. The techniques for demonstrating such involvement are available. If such an alteration occurred, Brodie would not term this effect a "potentiation." The simple additional experiment of allowing a pentobaritalized animal to regain his righting reflex, and then giving Urokon, whereupon the animal should return to sleep, would be further supporting evidence of the postulated competitive situation involving proteins.

Other experiments which would give data complementing those provided are the use of several other barbiturates, since some of these are known not to be bound by proteins and no potentiation by Urokon would be expected to occur. Also, the effects of other "potentiating" agents might be reconsidered in terms of protein binding. Two similar examples are the findings (a) that the compound known as SKF525A, if given simultaneously with decamethonium, causes an eight-fold increase in the toxicity of the decamethonium; and (b) that sulfa drugs bind with proteins, thereby releasing bilirubin, which may be absorbed and cause kernicterus, a central nervous system dysfunction, in some patients, many times in infants. It is of interest that several compounds of the SKF525A-type (chemically, this compound is β-diethylaminoethyldiphenylpropyl acetate), which are known to prolong the actions of hypnotics, analgetics and many other compounds, have generally been considered to do so by "increasing responsiveness of receptors to drugs," by "blocking excretion," or by "blocking biotransformation." The decamethonium experiment and the Lasser *et al.* hypothesis on Urokon potentiation by competitive albumin binding, suggest a possible refinement in the language with which mechanisms can be described, since combination with a specific molecule, even one in the blood stream which can probably only be involved with transport, is a firmer statement than any of the other common generalities mentioned above.

Although the iodinated radiopaque substance seems to have bound to albumin, and not to other plasma proteins, this protein may not be the only one which binds smaller molecules in other situations. Techniques are now available for studies of such combinations and competitions in isolated systems, or in model situations, wherein only the primary compounds are included. Actually, the various types of chromatography, spectrophotometry, electrophoresis, X-ray diffraction and other modern techniques hold great promise that the intimate details of the associations between many proteins and biologically important small molecules can be better understood.

A major point of this editorial is not to imply that Lasser and his colleagues have been derelict in their duty to science by not providing all the answers which might be worked out with available modern and elegant techniques, but to use the appearance of this report of interesting and well-executed work to call attention of the anesthesia fraternity to the "emerging" role of proteins in anesthesia research. As is usually the case, they are "emerging" largely due to the fact that techniques for studying them more satisfactorily are being developed. Another reason to call the role of proteins "emerging" is that the preoccupation of researchers with lipids and the greater solubility of anesthetic agents in them has finally been offset somewhat by the realization that such studies have not led to very satisfactory thoughts about the mechanisms of anesthesia, although the general predictive value of studies with lipids cannot be denied. Also, our realization that many of the small gaseous anesthetic molecules are transported in close association with blood proteins has led us to consider, in much greater detail, the actual roles proteins in blood and tissue are playing in anesthesia.

The recent clathrate and hydration hypotheses of Linus Pauling and Stanley Miller support the need for a greater number of protein studies, since both indicate that such complexes could not form under the conditions of clinical anesthesia without the intervention of some other stabilizing force, such as the type which might be supplied by a protein molecule. Lipid molecules could not, in most instances, supply this stabilizing force.

Several other factors about proteins should

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be kept in mind when considering their roles in anesthesia, or in other biological phenomena. One of these is the multiplicity of kinds of proteins. From the 22 amino acids which comprise the proteins in varying amounts and orders of combination, complex foldings of peptide chains are possible. Some of these provide a fairly rigid secondary structure and others provide a less rigidly held tertiary conformation or folding. The "solvent" for these proteins can influence profoundly the shape and thus the various potentials for chemical reactivity. "Solvent" is in quotes because it represents here the very complex mixtures of the biological milieus. Another system which must be studied more carefully is that involving the duplication processes for some proteins, the DNAs and RNAs. These may also be prompted to produce more of some kinds of proteins if the usual portions involved in important reactions are tied up by foreign molecules not necessarily having biological actions directly, like Urokon in the Lasser article. This field of study concerning the "inducible" proteins is in its infancy for most proteins found in mammalian systems, and the field promises to be of importance to anesthesia, as well as in the more obvious areas of genetics, embryology and cancer chemotherapy.

For those who may be influenced to look a little closer into these protein factors as they may be involved in the areas of their own researches, further words of caution are offered. The multiplicity of molecules falling under the general terms "lipid" and "protein" has been emphasized by Featherstone and Muehlbaecher. Not only is it necessary, or highly desirable, to know the nature of a protein factor being studied, it is also important to know its purity. Many "proteins" sold for such investigation are not homogeneous masses of identical molecules. Therefore, the source and purity of "proteins" used in *in vitro* studies should be clearly stated in all reports.

The generality of protein binding, although it has not been firmly established in many situations, may be of importance in explaining why some people have greater sensitivities to some drugs. Possibly they do not have normal amounts of binding proteins. Also, the shortness of the activity of succinylcholine has been attributed by Bovet partially to protein binding factors, rather than entirely to the rapid action of cholinesterases on the molecule. Furthermore, if such binding factors are shown to be important in competitions among several compounds for similar sites on proteins, these competitions may, in cases where several drugs are given simultaneously, lead to complex and puzzling responses.

A final factor to be kept in mind is that the blood proteins and their involvement in the binding of anesthetic molecules pertain primarily to the transport of anesthetics, but at the same time they provide a model of events within cells of the central nervous system, the primary locus of the anesthetic process. In these subcellular loci of the central nervous system, anesthesia must eventually be described. Such a description is being approached from the physiological side by many workers and from the molecular side by others. In view of all these factors mentioned, and since there is such an important acknowledged role for proteins in practically all types of biochemical reactions, studies involving proteins in anesthesiology should be encouraged as much as possible.

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