Some organic solvents, such as acetone and ethanol, have been used for protein fractionation.\(^1\)-\(^3\) Owing to their denaturing effect on proteins, the fractionation must be carried out at a low temperature, preferably at a temperature a little above the freezing point of the solution containing organic solvents. If this principle is followed, organic solvents, especially acetone and ethanol, will give an excellent result. From practical points of view, however, it is not an easy task to keep the material at an appropriate low temperature below 0°C during the whole course of preparation; this is particularly so at the step of centrifugation to collect formed precipitates.

For this and some other reasons, research workers prefer the salting-out method with ammonium sulfate. No doubt that ammonium sulfate is a very useful matter for protein fractionation. The most undesirable property of ammonium sulfate, however, is its concomitant increase in the ionic strength with progress of fractionation. Therefore, the protein assemblies that are liable to be disassembled at high ionic strengths are more or less decomposed by ammonium sulfate fractionation, and as a result, a considerable inhomogeneity of fractionated samples will take place. Furthermore, the protein fraction thus obtained, e.g., centrifuged pellets, contains a lot of ammonium sulfate, so that some procedure to eliminate it, e.g., dialysis, is necessary before being subjected to further experiments.

We have incidentally found that dimethyl sulfoxide (DMSO) is a useful organic solvent for protein fractionation. In addition to its advantages shared by organic solvents, the deteriorating effect of DMSO on protein functions has so far been shown to be less than not only those of other organic solvents but also that of ammonium sulfate.

**Materials and methods.** The special grade of DMSO produced by Wako Pure Chemical Industries Ltd. was used without further purification or modification. Purification by means of freezing-thawing did not give any result favorable for our purpose.

In this article the concentration of DMSO is expressed as per cent: x % means that x ml of DMSO is added to 100 ml of the original protein solution (total volume somewhat shrinks upon mixing DMSO with an aqueous solution, so the reference is made to the original volume).

**Results.** Since the space in this article is limited, we herewith describe only the outline of our experiences with DMSO fractionation. Further details including actual data will be presented elsewhere.

1) *Mixing heat:* DMSO produces a much higher mixing heat than does
ethanol or acetone. To be much worse, the freezing point of DMSO is fairly high, viz., 18.45°C, so that it cannot be cooled before use. Therefore, the addition of DMSO to the protein solution should be made very slowly and carefully.

One of the ways to avoid troubles due to the mixing heat is to take in advance a required amount of DMSO in the glass container, which is then cooled down to 0°C; after freezing of DMSO, the protein solution is put on frozen DMSO and then gently stirred; DMSO is gradually dissolved in the solution so that the temperature is easily controlled.

2) Temperature:—As emphasized also in other parts of this article, DMSO has much less denaturing effect on proteins than do any other organic solvents, but, even so, it affects protein functions irreversibly at higher temperatures, say, 30°C or above. Therefore, we must be careful that the temperature of the solution would not exceed a certain level, say, 10°C, upon mixing with DMSO.

As is well known, salting out of proteins is facilitated by raising the temperature, whereas the formation of precipitates by organic solvents is favored by lowering the temperature. In this respect DMSO is a typical organic solvent, being very sensitive to temperature, so the solution must be kept at a fixed temperature. It is most reasonable and practical to carry out all the procedures at 0°C. Exact control of temperature during centrifugation is a key to producing consistent results.

3) Time for precipitation:—Precipitation of proteins with DMSO proceeds more slowly than that with ammonium sulfate. After addition of DMSO, it is necessary to allow the solution to stand for 30 min. at least. A large part of precipitates is formed during this period, but a significant amount of precipitates is then gradually formed during following 10–15 hrs. One of the ways to promote precipitation is to lower the temperature of the solution below the working temperature and, after a while, warm up to the working temperature.

4) Ionic strength:—An important factor for protein fractionation with organic solvents is the ionic strength (the ionic strength in the presence of organic solvents, which reduce the dielectric constant of water, is difficult to define, but in this article, 'ionic strength' refers to that of the solution before the addition of organic solvents). At lower ionic strengths, say, less than 0.01, precipitates are hardly centrifuged down. With increase in ionic strength easily sedimentable precipitates are formed, the optimum ionic strength being 0.1–0.15. With further increase in ionic strength, the solubility of protein increases and the amount of sedimentable precipitates at the same DMSO concentration decreases. Even at such a high ionic strength as 1.2, however, most proteins could be sedimented if the concentration of DMSO is raised to 150%. This property may be utilized for separating a particular protein.

Unlike other organic solvents DMSO retains many chemicals in a solubilized state. This is true of strong salt solution. KCl is easily precipitated from its solution by ethanol or acetone, but 1.4 M KCl with an equal volume of DMSO does not form precipitates. Virtually no precipitate is formed from strong NaCl solution.

5) Concentrations of DMSO:—Roughly speaking, the precipitability of a protein in DMSO is parallel with its apparent molecular weight, i.e., an assembly of protein behaves as a single macromolecule, unless the complex is disassembled by DMSO. Since DMSO does not increase the ionic strength of the solution, the pattern of proteins fractionated with this material is considerably different from that with ammonium sulfate. For instances, smooth muscle myosin is more
precipitable with ammonium sulfate than is smooth muscle tropomyosin, viz., precipitation ranges are 20–28 g per 100 ml for the former and 30–36 g per 100 ml for the latter, whereas tropomyosin is more sedimentable with DMSO than is myosin at higher ionic strength, e.g., at 1.2, between 30–45% for the former and between 40–50% for the latter, though no clear difference is seen at lower ionic strengths, e.g., at 0.1, between 5 and 15% for both.

6) Miscellaneous:—Sodium dodecyl sulfate (SDS) is almost freely soluble in DMSO. This can be utilized for eliminating SDS from proteins, but it may indicate that SDS does not fully exert its unfolding effect on the protein conformation. Urea is also soluble in DMSO. It is not yet clear whether the characteristic effect of urea on protein structure can fully be exhibited in the presence of DMSO.

One of the advantages of the use of DMSO is the low salt content of centrifuged pellets. Unless DMSO itself is effective on the reaction to be tested, the pellet dissolved in due solution can be utilized without eliminating DMSO (DMSO exerts a more marked effect on the reactions of various protein systems than does ethanol or acetone, in contrast with its weak denaturing effect on proteins).

Discussion. Since we have not yet scrutinized the literatures, we are not confident that the observation stated in this article is a new finding. However, at least in our scientific community, it has not yet been mentioned that DMSO is an elegant material for protein fractionation. So it may be worthwhile to communicate this finding, though our study is still on a preliminary stage.

As researchers working on muscle proteins, some of which are apt to be denatured, we pay much attention to the deterioration of proteins. For the time being we are pursuing leiotonin, the factor to activate the actin-myosin-ATP interaction of smooth muscle without phosphorylation of myosin light chain, focusing on the role of 155 kDa proteins of bovine stomach and aorta. Both ammonium sulfate and DMSO give rise to almost the same yield of the activity on the first fractionation, but from the second fractionation, there has appeared a significant difference, DMSO being definitely superior to ammonium sulfate in retaining the activity.

Dioxane has been used as a fractionating material. We have not yet compared DMSO with dioxane carefully, but according to our few experiences, the deteriorating effect of dioxane appears to be stronger than that of DMSO (commercial dioxane contains some stabilizing reagent, which may be responsible for this undesirable effect).

Owing to the remarkable progress in chromatographic technique, the position of chemical reagents for protein fractionation has been lowered. However, many proteins, especially some muscle proteins, tend to deteriorate when applied to ion-exchange and/or affinity chromatographies. Furthermore, it is not easy to handle a large scale of sample by ordinary chromatographic procedures. Therefore, there has remained a plenty of room for the use of ammonium sulfate or organic solvents for protein fractionation. If the favorable properties of DMSO so far shown by our hands can be generalized for all proteins, the use of DMSO for protein fractionation will be a substantial contribution to biochemistry.

DMSO is a miracle reagent. It is utilized not only as an excellent solvent but also as valuable aids for many biological purposes, i.e., as the preservation material for cell and tissue culture, as the permeation-facilitating material in cellular physiology, and even as a remedy for some disease. Herewith, another interesting and useful property is added to this unique substance.
References