THE PREPARATION OF STEROIDS AS SUBMICRON PARTICLES

By

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DEDICATION

To my wife and my father in grateful appreciation of their help and encouragement.

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INTRODUCTION

It is often necessary or desirable to administer slightly soluble medicinal agents in the form of suspensions. These suspensions may be administered via oral or parenteral routes. When parenteral administration of a suspension is desired, it becomes exceedingly important to have the suspended particles in the finest state of subdivision obtainable. One class of slightly soluble compounds which are often used parenterally is that of the steroids.

This work was undertaken in order to devise a method by which steroids could be prepared as fine particles, with an average particle diameter less than one micron. Several avenues of approach were explored in the preliminary work in an attempt to determine the route by which this small particle diameter could be realized. Among the several methods studied, that based on rapidly freezing solutions of the steroids and removing the solvent by an adaptation of freeze drying technique was found to be the most promising. It was found that by this method it was possible to obtain uniform powders of extremely fine particle size.

To determine whether these powders were pharmaceutically desirable, and to gain a better understanding of their physical characteristics, preliminary investigation of the physical stability was also made. Exploratory studies of the effect of additives on the average particle size of the powders produced in this fashion were also carried out. These investigations showed that these powders possess very interesting and somewhat unusual properties.

NEED FOR STEROIDAL HORMONES AS FINE POWDERS

During the past decade great strides have been made in determining the role of steroid hormones both in normal body functions and as therapeutic agents. Since these compounds are usually only slightly water soluble, suspensions of them must be given when an aqueous, parenteral form is desired. These suspended particles are introduced into the tissues and remain embedded in these areas until gradually dissolved by the tissue fluids.

It has been shown that the larger the size of the crystals introduced into the tissues the greater is the irritation they cause to the tissues. The response to this irritation is the laying down of a connective tissue pad around the crystals to wall them off, which effectively reduces the amount of tissue fluid which can wash over the crystals. This results in a failure by the body to absorb enough of the hormone to reach a therapeutically active concentration, and as a result, the remaining hormone is gradually absorbed without fulfilling its intended function. By obtaining a more finely subdivided product, this condition may be relieved.

Other factors which favor the use of fine particles are the rate of solution and solubility, which

are greater for fine than for coarse particles. Consequently, the finer particles remain "in situ" for a shorter period of time. This works two ways in bringing about a more efficient use of the hormone administered. With an increased solubility the therapeutic level is more easily reached and since the particles are finer and more rapidly dissolved, the danger of encapsulation is reduced.

In considering the increased solubility of smaller particles, it is noted that under equilibrium conditions the amount of solute exceeding saturation is deposited on the larger crystals. However, in the body the dissolution is not carried out under equilibrium conditions since the tissue fluids bathing the local area are being constantly washed away and replaced.

From the pharmaceutical standpoint, the advantage of finely subdivided materials as suspensoids is obvious. The rate of sedimentation, which follows Stokes Law, increases with the square of the radius of the suspended particle. As a result, finer particles lend themselves more readily to the production of pharmaceutically elegant preparations and insure a uniformity of dosage from a multiple dose vial.

PAST WORK AND THEORY

In order to attack this problem in a logical manner, it was necessary to have a knowledge of the methods available for the production of fine powders. It was then possible to choose that method most applicable to the materials concerned.

METHODS OF PREPARING FINE PARTICLES

There are two routes through which the desired size of particle can be approached. The first method is that employing comminution of larger particles to produce the desired form. The second method approaches the desired size by the condensation of smaller particles (molecules and molecular aggregates) to produce the desired size particle. In this procedure there must be some method by which the growth can be arrested when the condensation has progressed far enough to produce the size of particle sought.

GRINDING METHODS

The attrition of materials to produce a powdered form has long been a procedure used extensively by pharma-

sists in the production of better preparations. Even in the days of the ancients, mortars and pestles were used for the powdering of crude drugs. As knowledge increased concerning use of medicinal agents, so did the attempts at producing more uniformly subdivided powders. At the present time there exist a wide variety of machines designed for the subdividing of materials used in the preparation of pharmaceuticals, but relatively few of these are applicable to the production of particle sizes within the range desired for suspensoids intended for parenteral administration.

The most widely used machines for the production of pharmaceutical powders by grinding can be classified into two types. (1) The first type makes use of a high speed rotor design and the second uses fluid-energy to accomplish the reduction of particle size. The mechanism of the high speed rotor type is similar to that of the hammer mill in which the reduction of particle size takes place by mechanical attrition between two hard surfaces. The fluid-energy type uses compressed air or high pressure steam to effect the reduction in particle size. The shearing of particles occurs due to both the force of the fluid and to the materials colliding with themselves and the walls of the container.

In machines of either type it is necessary that some means of size classification be included so that a

uniform, fine powder results. In this way the particles can be kept in the size reduction zone of the apparatus until they are as small as required in the finished product. This is accomplished by the use of intense centrifugal fields, whereby larger size particles are kept in the periphery of the size reduction chamber, while smaller particles with less centrifugal force are allowed to leave through specially adapted exits located centrally in the size reduction zone.

In this way, deviation of individual particles from the average particle size is held to a minimum. It is still found that the final product may contain 5-10% by weight of particles three to four times as large as the average size. Depending upon the substance being subdivided and the conditions of the operation such as feed rate, rotational speed, fluid flow rate, etc., the average size of particle achieved by these methods varies from 1 to 10 microns. Collection of the dust is usually made by two small diameter cyclone collectors in series or one cyclone collector in series with cloth bag type collectors, both ways bringing the efficiency of collection over 90%.

CONDENSATION METHODS

The condensation methods of producing fine particles may all be classed under the common heading, precipitation. For this reason an understanding of the

fundamental processes involved in precipitation is necessary. The mechanisms involved in this phenomenon are extremely complex and, consequently, much of the present day knowledge is purely qualitative. Quantitative treatment of the problem is being undertaken on a broad front at the present time. Perhaps in the future the mechanics of precipitation will be elucidated on a great enough scale to allow prediction of the conditions necessary to produce a desired result, but at the present time these conditions are found by exploratory studies, supplemented by the limited theorizing possible in the light of present day knowledge.

The size of crystal formed by precipitation of any substance is dependent upon the rate of formation of the crystal nuclei and the speed at which these embryonic crystals grow. These two properties are interrelated but are not dependent upon each other. For this reason, a separate discussion on each is necessary; yet to completely separate the two is an impossible task. This is borne out in the literature which shows that initial development of each phase of precipitation was started separately, with attempts at gradual merging of the two phenomenon into one discussion in more recent literature. However, the union is rather incomplete and unsatisfying.

Nucleation

Formation of Nuclei: Nucleus formation has been studied both in melts and in supersaturated solutions and the

observations made in both cases have been parallel, which indicates that common laws govern nucleation in both these instances. As a result, most discussions of this nature have used information obtained from melts and supersaturation studies interchangeably. This has led to a greater development of this phase than would have been possible, since nucleation is a phenomenon which lends itself to experimental study only with difficulty. The primary reason for this is the magnitude of the particles or agglomerates with which it is concerned.

The way in which these crystallization centers develop has long been a matter of discussion, there being considerable disagreement as to their exact origin. There are two ways in which the nuclei may be produced. In the first instance, nuclei may form spontaneously from the crystallizand by orientation and aggregation of a sufficient number of molecules. Crystallization initiated in this way is known as homogeneous crystallization. In the second type, known as heterogeneous crystallization, the nuclei originate at an interface such as at the surface of suspended, insoluble particles, or on the walls of the container. (2,3,4) It is only in rare instances that homogeneous crystallization can be demonstrated, and as a result, all crystallizations commonly encountered can be assumed to be of the heterogeneous type.

In the kinetic development and treatment of nucleation, homogeneous type formations are most easily considered. Since they correspond to the ideal situation, in which mathematical laws and formulations apply, many authors utilize this theory in their attempts to elucidate more completely the mechanisms of crystallization. (5,6,7,8)

Homogeneous Formation of Nuclei: The proposed mechanism of homogeneous nucleation is very simple. Within the liquid from which solid portions are to separate, spontaneous fluctuations occur. According to Dunning (6) these fluctuations begin as two molecules, then a third, fourth and so on to form into lattices of localized molecules until minute structures are formed of the same crystalline nature as the solid. These aggregates of the newly formed phase must reach a critical value in order to become stable. Up to the point of this critical value the centers may redisperse by dissolving, but beyond this point they grow as crystals. (9,10) Turnbull and Fischer (8) have shown that subcritical nuclei require free energy for further growth; while beyond the critical point, they grow freely with a decreasing free energy. It is only rarely that a nucleus will reach the critical value, since a long chain of favorable energy fluctuations is required.

Heterogeneous Formation of Nuclei: In contrast to the

advanced development of the theory of the homogeneous process, the actual mechanism of heterogeneous nucleation is still largely a matter of speculation. This is to be expected since explanations of observed phenomena are usually quite diversified until it is possible to accumulate enough experimental evidence.

Some thinking holds that the foreign particles may be isomorphic with the material studied or the surface may have a specific orientation of molecules or forces to enhance the aggregation of molecules of crystallizand. Still further work indicates the dependence may be more on the number and size of the foreign particles than on their nature. (4)

In addition to the influence of foreign surfaces, heterogeneous crystallization may be initiated by pre-existing nucleation centers in the crystallizand. These may be tiny blocks or crystal molecules of new phase (11,12) or they may be heterophase fluctuations which occur near the equilibrium point (melting point or solubility curve) to give rise to a heterogeneous system. (13) These fluctuations occur in the same manner that nuclei form for homogeneous crystallization, the distinction being that in this case, the nuclei are being formed at a time when they are thermodynamically unstable. Since these nuclei exist when the system itself becomes thermodynamically unstable in the process of crystallization, they serve as the initiators of the new phase.

The evidence supporting the view that crystallisation is commonly caused by foreign particles and/or pre-existing nucleation centers, is found primarily in "aging" experiments. In this type of study, attempt is made to show the dependence of the incidence of nucleation on the previous history of the system. If a correlation can be shown, the logical conclusion must be that these centers exist in the system and are affected by environmental conditions, because if nucleation occurred only via aggregation of molecules, previous treatment would not show any effect.

Tammann was probably the first to show these aging effects. (14) He showed that by superheating melts increasingly longer periods, the incidence of nucleation was greatly diminished. Richards, Kirkpatrick and Hutz(2), working toward the same end, heated samples to temperatures above the melting point, and found that the higher the level of heating, the further could the mass be supercooled before crystallization was observed, again indicating a reduction in the tendency to nucleate. They explained these findings on the destruction of overheatable nuclei which are formed by orientation and adsorption of molecules in the crevices of impurities. The harder such a nucleus is to destroy (more stable to heat), the less capable is it of projecting crystals into the liquid, hence slower growth and an observed aging effect. Egli and Zerfoss point out

that work at the Naval Research Laboratories at Washington D. C. also confirmed Tammann's work. (15) Working with extremely pure forms and systems, superheated nuclei were still observed, indicating that these are quite different from the superheatable nuclei due to the influence of foreign particles.

Opponents of the viewpoint that there exist pre-formed nuclei in any system, argue that the observed aging effects are due to insoluble impurities which are melted or dissolved on prolonged superheating. (15) The existence of superheatable nuclei is also questioned since it is out of harmony with the theory of lattice dynamics of Born and theories of melting as discussed by Mayer. The difficulties in proving or disproving this point are tremendous, a fact which is attested to by the difference of opinion which still rages.

Factors Influencing Nucleus Formation

Regardless of how the nuclei form, in a homogeneous or heterogeneous manner, certain factors have been shown to affect the rate of formation and the number of nuclei formed. The more important of these are the degree of supercooling of a melt or supersaturation of a solution, the viscosity of the system, the presence of foreign substances, and mechanical effects. These factors will be discussed in the following passages.

Supersaturation and Supercooling: The most important single factor in determining the rate of nucleation of a system is the degree of supercooling of melts or the supersaturation of solutions. These are analogous factors for the two systems and show identical relationships to their respective systems.

Working with melts, Tammann showed the effect of supercooling on the rate of formation of nuclei. (14) In this pioneer work, he showed that as the degree of supercooling was increased, the rate of formation of nuclei reached a maximum. Further supercooling beyond the maximum point resulted in a sharp decrease in the number of nuclei found, since in these regions, the effect of supercooling was being overshadowed by other effects, most notably that of viscosity. He also found a latent period between the melting temperature and the temperature at which grain formation began, which varied in magnitude with the individual substance tested. Similar studies on glycerol (4) showed the same effects on supercooling the melt, with an extremely rapid decline in nuclei number found after passing the maximum in the curve.

The latent period found in these investigations on supercooled melts corresponds to the metastable region of solutions as defined by Miers (16,17). In his studies, Miers showed that solutions remained stable in concentrations higher than those predicted by the solubility curve. After a certain degree of concentration, the solutions crystallize

spontaneously and the curve joining these concentrations when they are plotted vs temperature, he called the supersolubility curve. The area subtended between the supersolubility curve and the solubility curve he classed as the metastable region. Ting and McCabe have shown that two curves may be drawn beyond the solubility curve, the first indicating the point where nuclei are first observed and the second denoting the place where a pronounced heat effect and a copious evolution of nuclei are found. (17) Others have modified Miers concept to a passage from a state of slow crystallization to rapid crystallization.

In this metastable region crystallization can only be initiated by adding to the system a source of nuclei such as seed crystals. Beyond the metastable region the precipitation is spontaneous. All this work points to the existence of a supersaturation value at which the evolution of nuclei is at a maximum. The fact that as supersaturation increases the size of the critical nucleus decreases is also a contributing factor to this maximum. (3,21).

Viscosity: The second factor to be considered in a discussion of the rate of formation of nuclei is that of viscosity. Its effect has been shown by work with sucrose solutions (18), the studies on melts by Tammann and the study of glycerol. Alexander and Johnson discuss the rate of formation of nuclei. (19) It is brought out that

to form a nucleus, an aggregate must be formed from molecules of low kinetic energy, which explains the rise in nucleation rate on cooling. They further state,

> "The rate of nucleation might thus be expected to increase indefinitely as the temperature is reduced, but another factor is involved, namely, the translational and rotational movement of the molecules which is necessary for the formation of a nucleus. The rate of molecular movement will be approximately proportional to the reciprocal of the viscosity, and due to the viscosity increasing exponentially with falling temperature, it will fall off rapidly as the temperature is lowered. When this factor exceeds the previous one, the rate of nucleation falls again, giving a maximum in the overall rate-temperature curve."

Foreign Particles: The effect of foreign particles on the rate of nucleation has been studied as long as nucleation itself. Unfortunately, in the present state of knowledge, it is impossible to understand or explain the mechanisms whereby they exert their influence. As a result the work in this field has been primarily empirical, attention being focused more on what effect various substances have rather than on how they act. When the principles of nucleation itself have been more clearly elucidated, it will be possible to determine which processes in normal nucleation are being affected by foreign bodies.

Once again it was Tammann who struck the spark lighting the way for further observations of this type. In working with betol melts, he showed that quartz, emery and fused feldspar increased grain formation, while glass

powder reduced the nuclei number to zero. He also showed how impurities shifted the temperature at which nucleation reached its maximum. (14) Subsequent work has shown these effects to hold true not only for melts, but for supersaturated solutions as well. (4,11,20,21) The only generalization that can be made from all this work, is that high molecular weight compounds seem to be the most effective inhibitors of nucleation.

Mechanical Influences: The last factor to be considered in a discussion of nucleation is the effect of mechanical impact in the system. Vigorous stirring and collision of crystals in the solution with each other or the walls of the container may cause formation of new nuclei over and above the effect of fragmentation of existing crystals. (11,18) That mechanical impact has an effect in the promotion of nucleation has long been accepted, hence the very common laboratory procedure of scratching or striking the container holding the solution in an effort to induce crystallization.

There are other factors which have been demonstrated to affect nucleating systems in certain instances, but none of these is presently considered to be as commonly active as any of the factors discussed. For this reason, a consideration of these less common influences has been omitted from this discussion.

Crystal Growth

The consideration of crystallization from the point at which the nucleus has formed to the end of growth of the crystal is another field in which many workers have toiled their lifetimes. The quantitative development of this field has progressed at a slightly more rapid pace than in the case of nucleation, but the elusive, fundamental mechanisms are still beyond reach. Theories explaining how crystals grow have been proposed since the turn of the century, but none as yet has been able to fit all the observed data into its framework. (22)

In contrast to the studies of nucleation where a large amount of work was done with melts, it is found that observations of crystal growth have been made primarily on supersaturated solutions, since these lend themselves more readily to this study. The theoretical considerations are also dealt with in a more precise manner. None the less, the conclusions reached for supersaturated solutions can generally be applied to melts.

Theories of Crystal Growth: When a crystal grows from a supersaturated solution, the concentration of the solution in contact with the crystal surface is reduced, a concentration gradient is set up and the crystal is fed by diffusion down this gradient. The process of crystal growth may therefore be divided into two aspects - the

taking of solute from the solution by the crystal face, and the replacement of this solute by diffusion from the body of the solution. (23)

It is the first aspect of crystal growth, namely the deposition of solute on the crystal face, which offers the greatest resistance to complete development of the mechanism of crystal growth. Recent theories concerning the mechanism of the deposition of solute on the crystal may be classed into two groups - the adsorption-layer theories, and the theories making use of the fine structure of surfaces. (22) Although this distinction is made, attempts to bridge the gap between the two classes are meeting with some success, and it is very likely that the true mechanism of crystal growth is a hybrid of the two.

The adsorption-layer theories hold that a crystallizing particle only loses a portion of its latent heat on arriving at the crystal surface, but through this loss it is effectively bound to the surface. The particle is not held immobile on the surface, but has considerable freedom of movement laterally, sliding about over the surface like a two-dimensional gas. If this particle is taken in consideration with similar particles in its neighborhood, they constitute an adsorbed layer, which is taken as capable of attaining equilibrium with the solution in an instant. Frequent collisions between the

particles are to be expected and from some one or another inelastic collision, the germ of a two-dimensional crystal will be formed. This seedling attaches itself to the lattice and very rapid deposition to complete the lattice plane ensues. In this theory, the limiting factor is the rate of formation of the two-dimensional nucleus.

The theories which consider crystal growth from the standpoint of the fine structure of the surface are given more credance, the most notable advances being initiated by Kossel and by Stranski. (22) Their theories have a great deal in common, but the authors differ on one important point - Stranski feeling there may be a connecting link between his theory and the adsorption-layer theory, while Kossel does not regard this as feasible.

In these theories, the particles adding to the crystal structure are not free to move in a two-dimensional plane, but are fixed at the point of attachment. Once the initial "block" has been attached to start the new plane, the growth spreads as more particles are deposited, row by row, on the crystal surface. The limiting step in these cases is the rate at which the first particle deposits on the previous surface plane, the filling out of the new plane occurring very readily.

The descriptive character of both classes of attachment theories is seen to be very similar. It is in the mathematical treatment of the energy relationships

involved where the differences are noted. However, for the purposes of this report, evaluation of the theories on an energy basis is not required.

Factors Influencing Crystal Growth

As was shown for nucleation, several factors play important roles in modifying crystal growth. Since nucleation and crystal growth are very closely allied, it is to be expected for the most part that the same influences are active, namely the degree of supersaturation, the viscosity, and the effect of foreign substances. Again it is unfortunate that the bulk of the knowledge concerning these factors is qualitative and will of necessity remain so until the actual process of crystal growth has been more intimately determined.

Supersaturation and Supercooling: The greatest driving force for crystal growth is the degree of supersaturation. (4,11,23) Although the theories of crystal growth presented above minimize the effect of diffusion, it must be recalled that these only describe and explain the second aspect of crystal growth, the deposition of solid material onto the crystal surface. The rate at which solute is supplied to the surface, and the amount of solute in the layer of solution adjacent to the crystallizing surface, serve important functions in the rate of crystal growth.

In consideration of the counterpart of supersaturation, the supercooling of melts, it is found that if the rate of growth of the crystal be plotted against the degree of supercooling of the melt, a curve results which exhibits a maximum in much the same way as the supercooling curve for nucleation. The maximum is not as great, but is more prolonged than in the case of nucleation. (21,24) As a general rule, the maxima for the supercooling curves do not coincide, the maximum for crystal growth occurring at a temperature closer to the melting point.

Viscosity: The effect of viscosity is projected into the rate of crystal growth through the decrease in diffusion caused by the increase in viscosity. The velocity of diffusion is slowed down in inverse proportion to the viscosity, and the velocity of diffusion shows a direct relationship to the velocity of crystallization, both increasing or decreasing proportionately with viscosity changes. (20)

Foreign Substances: For a discussion of the effect of foreign substances on crystal growth, a wealth of experimental work is found. Probably of the greatest significance is the work of Mare and his co-workers who examined the inhibiting effect of dyes upon the crystallization

velocity. (19,25) It was found that dyes capable of coloring the crystal were effective in inhibiting and even stopping completely the growth of crystals. The degree of inhibition increased with dye concentration, which would be expected if the process were considered to be adsorption. Another indication that these dyestuffs functioned by adsorption is the fact that the rate of dissolution of the crystals was not affected. If these dyestuffs formed a sheath around the crystal, on transferring the crystal to fresh solvent, some diminution in the rate of dissolution would be expected.

Many other instances of inhibition of crystal growth by additives, especially high molecular weight colloids such as gums and gelatin, are found in the literature. (4,20,21,24,26,27,28,29,30,31) These findings are explained on the basis of adsorption of the additives onto the surface of the crystal, thus interfering with the normal growth, since even with colloids little or no effect on the rate of solution is noted. These foreign substances depressing crystallization velocity do not sensibly alter the velocity of formation of nuclei. There are also examples reported in which an augmentation of crystal growth occurred on the introduction of certain additives to the system studied.(24)

That the additives may be preferentially adsorbed by different faces of the crystal - for the most

part these exhibiting the fastest growth (21)-is indicated by the observations in which additives have changed the crystal habit. One example of this is the action of methylene blue on silver chloride crystals, from which irregular dendritic crystals appear rather than the symmetrical crystals which are usually formed by silver chloride. (28) Another example is the crystallization of sodium chloride as octahedra instead of cubes under the influence of urea and certain dyes. This is explained by Alexander and Johnson who state,

> "Sodium chloride, for example, normally crystallizes as cubes owing to the high energy of the octahedral faces (consisting of Na^+ or Cl^- ions exclusively), relative to the cube faces (composed of alternate Na^+ and Cl^- ions), which leads to deposition of material on the latter exclusively. If, however, the relative energies of the surfaces can be reversed by adding an adsorbate taken up preferentially by the octahedral faces (as with urea and certain dyes), then octahedra will be formed." (19)

As another example of the role impurities and additives can play in the inhibition of crystal growth, the production of colloidal cholesterol suspensions - a subject very closely allied to this investigation - may be traced. In 1908, Porges and Neubauer prepared colloidal suspensions of cholesterol by rapid precipitation from acetone solutions. (32) Other investigators prepared cholesterol in colloidal suspensions by precipitation from acetone (33), alcohol (34,35) or acetone-alcohol (36) solutions even as late as 1930. However, it was shown in 1925 (31), and

again in 1936 (37) that pure cholesterol could not be prepared as a colloidal sol by these methods, even in small concentrations. This pointed out that some impurity or combination of impurities in the cholesterol samples commercially available at that time was working to inhibit the growth and aggregation of the cholesterol crystals formed on precipitation from its solutions.

From the foregoing discussion of the underlying factors governing the process of precipitation, it may be concluded that optimum results for the smallest crystal size can be attained if a high degree of supersaturation can be produced in a relatively viscous medium in the presence of a suitable protective agent. That this situation is difficult to achieve is brought out by the relatively few attempts made by colloid chemists to produce colloidal suspensions in this manner. To solve a given problem would require hit or miss techniques and combinations, with very little theorizing possible to avoid the "dead end" explorations which would inevitably result.

VARIATIONS OF THE PRECIPITATION TECHNIQUE

Rapid Freezing - Sublimation Drying

Rapid Freezing: An approach to the precipitation of substances as fine particles was made from another direction in this investigation. Since both nucleation and rate of

growth fall off as the temperature of the system is decreased, reaching negligible values on sufficient cooling, the amount of condensation can be controlled by temperature regulation. Findley states,

> "Since the temperature at which the spontaneous formation of crystal nuclei has its maximum value is, in general, below that at which the velocity of crystallization is a maximum, it is possible, by rapid cooling, to pass through the temperatures of maximum crystallization velocity and maximum formation of nuclei, and to obtain the liquid at a temperature at which the velocity of crystallization (and also of crystal nuclei formation) becomes negligible." (24)

Use of this property of crystallizing systems was made to facilitate the attainment of fine particles.

Although the statements concerning supercooling were confined primarily to observations on melts, supersaturated solutions could safely be assumed to exhibit the same property. There is an added complication in the case of solutions in that two substances are involved, but if the rate of cooling is rapid enough and the extent of cooling sufficient, the difficulties, for the most part, should be obviated. It is logical to assume that the cooling should be extended to such a temperature that both components of the system are frozen. In this case the viscosity of the system is so extreme that growth of the crystals should be zero, since diffusion should be negligible. Therefore, the total condensation which should occur up to the point of obtaining the frozen mass, would

occur during the short period of time between the reaching of the supersaturation level at which spontaneous nucleation occurs and the freezing of the solvent.

Sublimation Drying: At this point the problem becomes that of removing the solvent from the frozen mass. This involves the sublimation of the solvent, so the use of the principles of drying by sublimation are indicated. The theoretical aspects of this procedure are simple, yet no published reports of the application of this technique to any systems other than aqueous were found.

The fact that ice can be made to sublime at a rapid rate, rapid enough to maintain itself in the frozen state due to the absorbing of heat in the evaporation process, has been known since the times of early scientific work. (38) However, it wasn't until the twentieth century that application of this known principle was made in the field of drying. Within the past decade, the strides forward have been tremendous, so that today it is such a common procedure that freeze dried products can be found in almost any household.

The great impetus to the development of freeze drying was supplied by the necessity of producing blood plasma in a stable, easily handled form during the second World War. Another early advancement came from the application of freeze drying to the production of stable

penicillin preparations. Since these early, large scale investigations, the process has been adapted to use on many pharmaceutical preparations and food products, such as frozen fruit juices and powdered coffee. (39)

Of particular interest to this research is the fact that on drying by sublimation there is only minimal coagulation of the solid residue since the solute particles are virtually locked in position as the solvent evaporates. (38) This means that the growth of the fine particles produced by the rapid freezing, is restricted during the removal of solvent. On the other hand, however slight the growth may be, the amount of growth relative to the initial size produced in the frozen mass may be of significance. This would have to be determined through experimental procedures.

The rate at which the drying proceeds is dependent upon the pressure differential existing between the vapor pressure of solvent in the sample and the vapor pressure of solvent in the condenser, which serves to trap solvent vapors, thereby maintaining the vacuum in the system. These vapor pressures are in turn dependent upon the temperatures of the subliming sample and the condenser. On this basis, the temperature at which the product should be dried determines the temperature at which the condenser should be maintained in order to keep the pressure differential at the optimum value for rapid drying.

This can best be explained by citing an example. In drying blood plasma, which should be maintained at -18° C. for drying, it was found that if the condenser was kept at -35° C., the drying was completed in 21 hours, whereas, if the condenser temperature was maintained at -40° C., the drying time was 87 hours. In the latter case, it was found that the initial evaporation, due to the higher pressure differential, was so rapid that the temperature of the sample fell to -32° C., at which point the system reached equilibrium and the temperature of the sample remained constant. The pressure differential in the first case is optimum, while the pressure differential existing between the sample at -32° C., and the condenser at -40° C. is not, hence the increased drying time. (38)

Unfortunately for this undertaking, previous work on the principles of freeze drying has been done only on aqueous systems. Since in this work the solvents removed by sublimation were organic, the procedures followed were, of necessity, worked out by exploration, each success or failure leading to the development of the final technique by which the freeze dryings were accomplished. As a result, the efficiency of the method, as employed, is questionable.

Spray Drying

One other condensation method of obtaining substances in a fine state of subdivision which merits consideration is the process of spray drying. Since the basic

factors underlying the precipitation processes may play a role in determining the physical properties of the products obtained by spray drying, this process can be considered to be a distant modification of precipitation.

The principle of spray drying is quite simple. In the process, a highly dispersed state is introduced into a high temperature, circulating gas zone. Because of the extremely large total drying surface exposed to the hot gas, the drying time of the dispersed droplets is measured in a fraction of a second. The solid portion of the droplet is left in the form of a spherical particle, solid or hollow, depending upon the material, the feed conditions and the drying conditions. (40)

Design of Spray Driers: Seltzer and Settelmeyer have classified spray driers according to their design into five categories. (41) The design is dependent on the direction of gas flow and its relation to the direction of spraying. Each design has certain advantages for the spray drying of specific materials, the gas flow being designed to bring about the most efficient drying of the material introduced. In the chemical industry, the design of most favor is that in which the hot gases circulate in a rotary motion down around the atomizer, co-current with the mist being produced. The popularity of this type of gas flow in the chemical industry is in part explained by

the fact that the atomizer of choice in this industry is the centrifugal disc type. With this type of atomizer, the co-current, rotary gas flow is the most efficient for drying. The hot gas, usually air, is produced either by direct heating or by passing through steam heated coils.

Atomizers: The atomizers used are of two types, the centrifugal disc type, and the nozzle type. In the case of the former, the dispersed state is produced in the following manner. The liquid is extended in thin sheets on the disc, these sheets being discharged into the surrounding hot gases at high speed from the periphery of the disc, which is rotating very rapidly (up to 50,000 r.p.m.). For top efficiency, the liquid should reach disc speed before leaving the disc because at this speed, the surrounding air becomes a dense wall of great relative inertia, thus aiding to pulverize the fluid. (42)

Pressure nozzles effect atomizing by forcing the liquid through a small orifice under high pressure. A variation in design of the nozzle is the two fluid nozzle, which employs air or steam as the atomizing fluid. These operate at lower pressures, but the efficiency of this type of nozzle is reduced at high capacity. In the high pressure nozzle, the spray characteristic depends upon the pressure and the orifice size, and as a result is fairly inflexible. The greatest drawback to both types of nozzle atomizers is

that they show a tendency to plug, especially when run at high capacities.

Factors Influencing Particle Size: It is generally believed that centrifugal disc atomizers give rise to the most uniform particle size distribution. The particle size produced by nozzle atomizers is approximately inversely proportional to the square root of the pressure, while with the centrifugal disc type of atomizer, the particle size varies inversely with the disc speed. It is also a function of the solids content of the slurry being dried, liquid viscosity, liquid density, and feed rate, increasing with these as they increase. (40,41) By adjustment of these factors, particularly nozzle pressure or disc speed, solids content of the slurry and feed rate, the particle size obtained can be controlled to a certain extent.

As the particles produced become smaller, the problem in separating them from the gases becomes increasingly difficult. The collection is made in the same manner as for the grinding techniques discussed previously, using small diameter cyclone collectors and cloth bag filters. Wet scrubbers, which act by recapturing the smallest particles in a liquid, usually the solvent of the slurry, minimize the loss of material as dust, but the liquid must be recirculated through the drier to

recover the solid. (41) Where extremely fine particles are desired, it would be necessary to rerun the material several times before the desired fineness could be obtained in an efficient operation (from the standpoint of percentage conversion to fine particle size).

PLAN OF STUDY

The foregoing discussion of the methods available for the production of fine particles makes it possible to select that method which shows the most promise for the successful completion of the intended work. In view of the possibilities of success, it was decided to focus the main attention of this work on the process of rapid freezing of solutions of the compounds with which this research is concerned.

Additional work, entirely of an exploratory nature, was done in the field of protective crystallization with the hope of finding a technique where the proper blend of controlling influences would make it possible to precipitate these steroid compounds as crystals of the desired size. In addition, several samples of cholesterol were spray dried using a Bowen Laboratory Model spray drier, from which an indication of the merits of this method could be obtained.

As an indicator of the particle size produced by the rapid freezing-sublimation drying technique, the specific surface area of the powders produced was used. By altering one condition of the procedure at a time, the value of the specific surface area was determined as a

function of:

(a) Concentration of solute in the frozen solution,

(b) Refrigerant used to freeze the solution,

(c) Sample temperature during drying,

(d) Solvent used for the frozen solution, and

(e) Rate of cooling of the frozen solution.

In addition, the effect of various additives on the particle size of the powder produced was determined. These substances were dissolved in the solution of the steroid prior to freezing.

The stability of the product obtained by this method was determined as a function of temperature. In this study, the reduction in specific surface area of the sample, which occurred on heating for a specified period of time, was determined. For one sample, this was determined at three elevated temperatures, while for another, the decrease in surface area which resulted from standing at room temperature was measured.

EXPERIMENTAL

PART I. THE PREPARATION OF STEROIDS AS FINE PARTICLES

The experimental procedures herein described were performed with three steroids, cholesterol, pregnenolone, and Δ4-Androstene-3,17-dione, with the principle emphasis placed on the reaction of cholesterol to these procedures. This was done since it was felt that cholesterol, being the mother compound of the class, would exhibit properties common to a greater percentage of the steroids. With suitable modifications, however, the procedures used could be applied to any of the steroids.

In the initial stages of this study exploratory work was undertaken both in the field of precipitation by interchange of solvents and precipitation by the rapid freezing method. These preliminary investigations were carried out concurrently. The method employed which gave rise to the largest specific surface areas for the powders produced was the rapid freezing-sublimation drying method. Because of this, greatest attention was focused on this procedure, the effect of modifications of the procedure on the final product - which also gave an indication of the flexibility of the procedure - and the physical

properties of the final product. Work performed along the lines of solvent interchange precipitation and spray drying was not as detailed.

PRECIPITATION BY SOLVENT INTERCHANGE

The investigation of the procedure of precipitation by changing the solvent never advanced beyond the exploratory stage. After the initial studies it was necessary to limit the research to the study of one method. At the time the decision was made, the rapid freezing-sublimation drying procedure seemed more promising, and consequently, work on precipitation by changing the solvent of the steroids was abandoned.

Theoretical Considerations

The theory underlying this type of precipitation has been discussed in the preceding portion of this report. The primary requirement to succeeding in precipitating the steroids as fine particles is the production of a highly supersaturated system instantaneously. The only practical way to achieve this state is to interchange solvents by adding a solvent in which the steroid is highly insoluble, but with which the initial solvent is miscible. The protective agent used plays a secondary role in this process, but its importance is not to be discounted.

Procedure and Results

In all the studies of solvent-pair precipitation as a method of obtaining fine particles, solutions of pregnenolone in various solvents were used. These solutions were precipitated in aqueous solutions of protective substances. Several variations were attempted. In addition to dropping the solution into the aqueous phase, these solutions were also sprayed into the aqueous phase using a DeVilbiss No. 121 atomizer. Further, the solutions were dropped or sprayed into rapidly stirred aqueous solutions of protectants which were at room and at elevated temperatures.

In the first investigations one percent solutions of pregnenolone in a variety of solvents were dropped into 0.5% solutions of polyethyleneglycol monostearate 400 (PEGS 400) in water. A ratio of one part of pregnenolone solution to five parts of aqueous solution was used. The solvents for pregnenolone which were employed were butyl cellosolve, methyl cellosolve, diethyl cellosolve, tetra-hydro furan, dioxane, 95% ethanol and acetone. The crystal size of the suspended particles produced was measured by examination under a microscope fitted with an ocular micrometer attachment to give approximate dimensions. In these precipitations needle-shaped crystals were produced which were 1 to 8 microns in width and 8 to 10 microns in length. With diethyl cellosolve

and dioxane, rounded crystals 2 to 3 microns in diameter were also observed, but these crystal forms were by far in the minority.

For further precipitation studies more diversified systems were used. The conditions for these studies, along with the microscopic observations, are shown in Table I.

The microscopic observations revealed that the suspended particles were larger than the size which was desired in this work. Although these experimental studies represent only a minute fraction of the possible combinations and techniques which might be used, the failure to discover any promising signs or indications that might delineate further lines of study led to the abandonment of work on this type of precipitation.

RAPID FREEZING-SUBLIMATION DRYING STUDIES

Theoretical Considerations

In the rapid freezing-sublimation drying method, the most important single factor in controlling the particle size of the steroid as it exists in the frozen mass, is the rate at which the solution is frozen. The rate at which the cooling occurs depends upon the heat transfer through the solution to the freezing refrigerant. If the heat transfer were great enough, the freezing would be instantaneous, in which case the steroid would be distributed throughout

TABLE I

PROTECTIVE PRECIPITATION STUDIES WITH PREGNENOLONE

Pregnenolone Soln. Solvent	Conc.	Aqueous Solution Protectant	Conc.	Conditions	Observations
Diethyl Cellosolve	1%	PEGS 400*	0.5%	Sprayed	Crystals varied in size, grew larger on standing.
Acetone	1%	PEGS 400*	0.5%	Sprayed, aqueous at 35° C.	Long needles.
Diethyl Cellosolve	1%	PEGS 400* NaCl	0.5% 0.9%	Sprayed	Rounded crystals, 5 to 10 microns in diameter.
Dioxane	0.5%	Gelatin	0.2%	Sprayed	Small crystals, almost round.
Diethyl Cellosolve	1%	Arabic Acid	1%	Sprayed	Rounded particles.
Acetone	0.5%	Arabic Acid	1%	Sprayed	Rounded particles.
Acetone	0.5%	Arabic Acid NaCl	1% 0.9%	Sprayed	Needles approximately 1 x 5 microns.
Diethyl Cellosolve	1%	Arabic Acid NaCl	1% 0.9%	Sprayed	Large clumps and rounded particles.
Diethyl Cellosolve	1%	Arabic Acid Dextrose	1% 5.4%	Sprayed	Large clumps and smaller particles.
Diethyl Cellosolve	1%	Arabic Acid Dextrose	1% 5.4%	Sprayed from below surface.	Clumps and some smaller particles.

TABLE I (Cont.)

Pregnenolone Solvent	Soln. Conc.	Aqueous Solution Protectant	Conc.	Conditions	Observations
Diethyl Cellosolve	1%	Arabic Acid dextrose Diethyl Cellosolve	1% 6.4% 1%	Sprayed	Clumped masses.
Acetone	1%	PEGS 400*	0.5%	Sprayed and stirred rapidly.	Range of particle sizes.
Acetone	1%	PEGS 400*	0.5%	Sprayed, stirred rapidly. Aqueous solution at 60° C.	Large crystals.
Acetone	1%	PEGS 400*	0.5%	Stirred rapidly, added as small drops	Needles 8 x 8 microns.
Acetone	1%	PEGS 400*	0.5%	Stirred rapidly, added as large drops	Large needles.
Diethyl Cellosolve	1%	PEGS 400*	1%	Stirred rapidly, added as small drops	Small crystals which grew.
Acetone	1%	Arabic Acid	1%	Stirred rapidly, added as small drops.	Large needles.
Ethyl ether	1%	PEGS 400*	0.5%	Stirred rapidly, added as small drops.	Small rounded particles and needles.

* Polyethylene glycol monostearate 400

the solvent as molecules or aggregates of several molecules. Practically, this can never be achieved, nor would it be desirable, since on sublimation drying, the molecules and molecular aggregates would volatilize off with the solvent.

Two factors which affect the rate of heat transfer and which can be controlled, are the mass of solution to be frozen and the temperature of the freezing medium. As the mass to be frozen decreases, it can be expected that the time required for it to freeze solid will also decrease. In the normal procedure followed in this work the solutions were frozen in the form of droplets, which allowed the cooling to be rapid enough to give small particle distribution of the steroid in the frozen mass, and in which form the frozen solution was easily handled.

The temperature of the freezing medium was regulated by the choice of medium. In choosing the refrigerating medium it was necessary to consider the subsequent treatment of the frozen mass. Since it was intended that the solvent of the solution be removed by sublimation, the refrigerating medium had to be one which was also volatile, so that it could be removed before the solvent. For this reason, the freezing refrigerants chosen were those which boiled at a temperature below the freezing point of the solvent, and which were readily available. The refrigerants chosen were liquid air and liquid nitrogen. In this study, no attempt was made to

freeze the solution by dropping it onto a chilled surface.

In one way the use of the low boiling type of freezing refrigerant might be inferior to other methods of freezing. When a substance such as liquid air or liquid nitrogen is used, the heat transfer from the solution to the refrigerant is retarded because a cushion of air or nitrogen gas, respectively, is produced between the surface of the droplet and the liquid surface of the refrigerant. The heat transfer to and through the cushion of gas is very low. However, in this research, the effects of this retardation of cooling were not evident because the objective of the problem was to complete freezing of the solution in such a period of time as to give rise to the desired particle fineness, rather than to achieve instantaneous freezing.

A decided advantage in the use of these agents as freezing media is that they are very easily removed from the frozen sample in the same apparatus in which the sublimation of the solvent is carried out. This means that the process of rapid freezing and subsequent removal of solvent from the frozen state can be carried out with a minimum of handling. As a result, the procedure is simple and easy to follow.

Procedure

Rapid Freezing & Sublimation Drying: The first step in the procedure as employed in this laboratory was the freezing of the solution. The solution was prepared by weighing out the desired amount of steroid and adding 100 ml. of solvent. For example, a 5% solution was prepared by adding 100 ml. of solvent to 5 grams of steroid. The solution was then filtered and transferred to a 125 ml. separatory funnel of the narrow taper design. The freezing medium was placed in a wide-mouth, pint Dewar flask and the solution allowed to drip directly into the refrigerant at such a rate that discreet droplets of solution were formed before they came in contact with the freezing liquid.

The next step in the process was the sublimation of the solvent from the frozen mass leaving the finely subdivided steroid behind. This operation was carried out in the freeze-drying apparatus shown in Figure I. A Cenco Fressovac vacuum pump was used as the vacuum source with this apparatus.

The frozen solution was transferred to the previously chilled sample tube with an excess of freezing liquid, and the sample tube placed on the drying apparatus. Both Dewar flasks were filled with liquid air and set in place. Then, under the influence of the vacuum, the excess freezing medium was evaporated and the sample was degassed.

When the pressure of the system had been reduced to the point where a blue discharge from a Tesla coil was observed, the Dewar flask containing liquid air was removed from the sample and replaced with one containing the desired cooling medium to keep the sample from melting during removal of the solvent. The cooling medium varied with the conditions of the experiment, being either an acetone-dry ice mixture, or a cold air bath produced by pre-cooling a Dewar flask with liquid air. With all the cooling baths employed, glass wool was loosely placed around the top of the Dewar and pushed down slightly into it to act as an insulating material.

During the drying it was necessary to replenish the liquid air in the Dewar flask around the condenser twice a day. By limiting the size of the sample to 25 ml. of solvent, it was found that the original cooling baths for the sample survived (cooled by evaporation of the solvent) and functioned to keep the sample frozen during the period of drying. When a larger sample was run, the originally prepared cooling bath for the sample was not sufficient, but had to be renewed midway during the run to keep the frozen sample from melting. Because of the possibility of loss of an entire sample by this method, the samples were limited to 25 ml. of solvent, which permitted completion of the drying with the original sample refrigerant.

SUBLIMATION APPARATUS

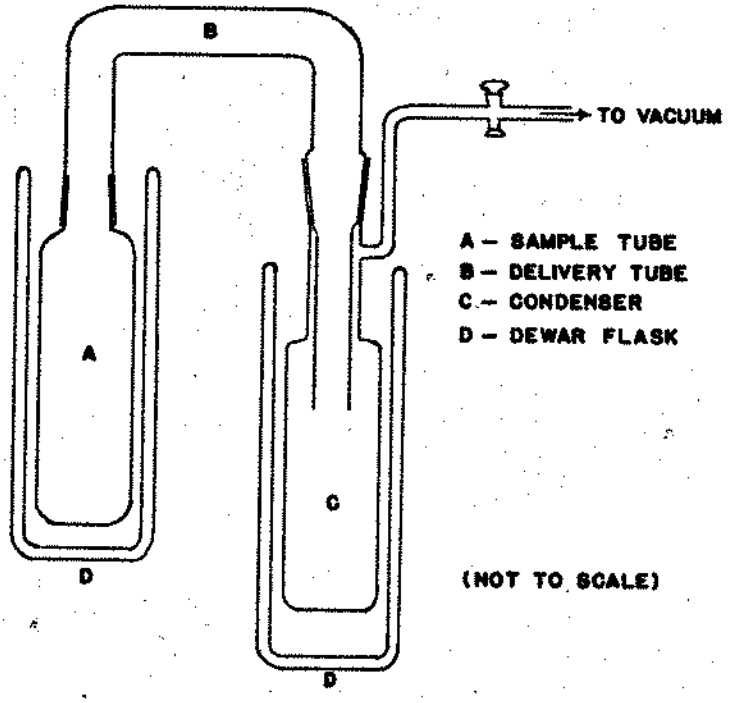

FIGURE I

Measurement of Surface Area: Since the steroid particles produced by the rapid freezing-sublimation drying technique were of such small dimensions, the most accurate method for the determination of their size, available in these laboratories, was the measurement of their specific surface area. The determination was made through the application of the Brunauer, Emmett and Teller method of nitrogen adsorption at low temperatures. The apparatus used and procedure followed were as described by Swintosky, et al. (43,44,45)

In these analyses, the amount of nitrogen adsorbed on the surface of the particles, which are cooled to the temperature at which liquid nitrogen boils, is determined. If these powders display sigmoid shaped adsorption isotherms, the adsorption occurs in monomolecular layers and is described by the equation:

$$\frac{P}{V(P_o - P)} = \frac{1}{V_m C} + \frac{C - 1}{V_m C} \frac{P}{P_o}$$

where V is the volume of gas adsorbed per gram of adsorbent at pressure, P, at a temperature at which the vapor pressure of the liquefied gas is P_o. V_m is the volume of gas in cubic centimeters (converted to Standard Temperature and Pressure) required to form a monomolecular layer on a gram of adsorbent, and C is a constant which is related exponentially to the difference between the heat of liquefaction and the heat of adsorption of the adsorbate.

The apparatus is so designed that P and V can be

determined for a given sample. If the value of P_o is known, V_m and C can be obtained from the slope and intercept of the line produced by plotting $\frac{P}{V(P_o - P)}$ versus $\frac{P}{P_o}$. This mathematical evaluation of the data has already been described in the literature. (43)

One modification of the procedure was introduced in this work. In the Swintosky procedure, when the sample tube containing the sample is attached to the apparatus, the volume of the system is unknown. The total volume must be measured with helium gas, being calculated from the data obtained by measuring the pressure changes in the closed system which occur when the volume is altered in known increments. By subtracting the volume of the calibrated portion of the system from the total volume determined with helium, the volume of the attached sample and tube is found. This method may give rise to considerable error, especially when larger sample tubes are used, since the accuracy of the method is on a percentage basis. This means that if the volume determined with helium were at a minimum, a much greater absolute accuracy could be achieved.

In the procedure followed in this research, advantage has been taken of this fact to give a more accurate measurement of the volume of the system with the sample and tube in place. To keep the volume of the system at a minimum for determination with helium, the

sample tube was filled with a known weight of mercury. The temperature of the mercury during the calibration was observed so that the volume of mercury in the sample tube could be calculated. After the volume of the calibrated portion of the system was subtracted from the total volume determined with helium, the volume of the mercury in the sample tube was added. The resulting volume represented the contribution of the sample tube, when in place, to the total volume of the system.

To find what the contribution was when the sample tube contained the steroid, it was necessary to calculate the volume of the steroid sample. Since the precise densities of the steroids were available (46), the calculation was simple. The volume of the steroid sample was subtracted from the sample tube volume to obtain the desired figure which also represented the volume of the system outside of the calibrated zone.

A knowledge of this volume is necessary because a certain portion of the nitrogen gas will occupy this space during a surface area determination. In addition, this volume represents that portion of the system which is kept at the boiling temperature of liquid nitrogen. Since the temperature in this portion of the system is so low, the concentration of nitrogen gas will be greater; consequently, in order to accurately calculate the amount of nitrogen gas occupying this portion of the system, the volume must be known exactly.

Program of Study

To study the effect of concentration of steroid in the frozen solution, the effect of the refrigerant used to freeze the solution, and the effect of sample temperature during drying, the following samples were prepared:

(a) 1, 2½, 5, 10 and 15% solutions of pregnenolone in chloroform, frozen in liquid air, using an acetone-dry ice mixture to cool the sample during drying.

(b) 1, 2½, 5, 10, 15, 20 and 40% solutions of cholesterol in chloroform, frozen in liquid air, using an acetone-dry ice mixture to cool the sample during drying. Check samples of 5, 10, 15 and 20% solutions were prepared.

(c) 5, 10, 15, 20 and 30% solutions of cholesterol in chloroform, frozen in liquid nitrogen, using an acetone-dry ice mixture to cool the sample during drying. Check samples were run on the 5, 10, 15 and 20% solutions.

(d) 5, 10, 15, 20 and 30% solutions of cholesterol in chloroform, frozen in liquid nitrogen, using a cold air bath to cool the sample during drying. Check samples on the 5, 10, 15 and 20% solutions were prepared.

(e) 10 and 20% solutions of cholesterol in chloroform, frozen in liquid air, using a cold air bath to cool the sample during drying.

The effect of concentration of solute was seen by comparing the differences in the samples of the same category. By cross comparison between the cholesterol

samples frozen in liquid air and those frozen in liquid nitrogen, the effect of the freezing medium was shown. Variation of sample temperature during drying was shown to have an effect by comparing the samples run using an acetone-dry ice mixture with those using a pre-chilled Dewar flask as cooling agents for the sample. That there is a significant difference in temperature of the samples under the influence of these cooling media is shown by the marked difference in the drying time for the samples. Those using a cold air bath as sample refrigerant dried in approximately thirty hours, while those samples cooled with an acetone-dry ice mixture required forty-eight hours to dry.

The effect of the solvent used for the steroid was explored by preparing duplicate samples of 10% cholesterol in carbon tetrachloride, frozen in liquid air, using an acetone-dry ice mixture to cool the drying sample. In addition, duplicate samples of Δ4-Androstene-3,17-dione were prepared from 5% solutions of this steroid in carbon tetrachloride using the same conditions as for the preparation of the cholesterol solutions in carbon tetrachloride. Cross comparison of the 10% solutions of cholesterol in carbon tetrachloride with identically treated 10% solutions in chloroform gave the most information.

In an attempt to determine the effect of the rate of cooling on the size of particle produced, three 10%

solutions of cholesterol were atomized into liquid air using a continuous air stream with a DeVilbiss atomizer No. 12l. In removal of the solvent from these samples, acetone-dry ice mixtures were used to prevent melting of the sample.

Finally, a number of samples were prepared from solutions to which various additives had been added. The first group of additives was used to explore the effects shown in the resulting powder which could be compared to a similar powder, prepared without the additive. The second group of additives was employed in an effort to reduce the particle size of the resulting steroid powder. Due to the nature and the amount of the additives used, these samples could not be analyzed for surface area, hence an exact measurement of the effect was impossible.

The additives fitting the first category were absolute ethanol, absolute methanol and a mixture of 50% Tween 20, 40% Tween 40 and 10% Tween 81. In all these cases, 10% solutions of cholesterol in chloroform were used. The additives were used in the following approximate concentrations in the frozen solutions: (a) absolute ethanol 0.1%, (b) Tween mixture 0.1%, and (c) absolute methanol 10%. Duplicate samples were run in all cases to check the results obtained.

Two additives of the second type were employed. The reason for using these additives was the hope that they would serve to prevent the larger aggregates of the

steroid from forming, both during freezing of the solution and during sublimation of the solvent. Of the two agents used, caffeine and PVP (polyvinylpyrrolidone), the PVP seemed to be better suited to the desired function. Being a long chain polymer, soluble in chloroform and water, it was hoped the particles of steroid would be enmeshed in the molecular framework and consequently, the size of the particles of steroid would be confined to the colloidal range. The functioning as a protective framework was felt to be especially important during the drying procedure since particles of the desired size would show high vapor tendencies and as a result, an increased tendency to agglomerate as the solvent was removed by sublimation. The ability of caffeine to fulfill this purpose was felt to be markedly inferior to that of PVP.

The caffeine samples were prepared from chloroform solutions which contained 5% caffeine with 1% cholesterol, and 5% caffeine with 0.5% cholesterol. Both of these samples were processed using a sublimation unit as shown in Figure 1.

The following samples were prepared using polyvinylpyrrolidone as the additive (concentrations indicated refer to the solution which was frozen):

½% PVP with 1% cholesterol
1% PVP with 1% cholesterol
5% PVP with 1% cholesterol
5% PVP with 0.5% cholesterol
6% PVP with 2% cholesterol
10% PVP with 2% cholesterol
5% PVP with 1% Cholesterol and 0.25% Tween mixture
10% PVP with 4% cholesterol and 0.5% Tween mixture

Some difficulty was experienced with the sublimation drying of these chloroform solutions containing polyvinylpyrrolidone.

Polyvinylpyrrolidone is apparently soluble in chloroform by virtue of association through the hydrogen in the chloroform molecule, which is capable of forming a hydrogen bond. The association complex which is formed reduces the vapor pressure of the chloroform in the frozen mass to such a degree that the vacuum developed in the apparatus diagrammed in Figure I is not sufficient to cause sublimation.

Because of this reduced vapor pressure, it was necessary to design a new sublimation drying unit for use with these samples. This unit, diagrammed in Figure II, made use of a Cenco Pressovac vacuum pump fitted with an oil diffusion aspirator, a large bore conduit system and a second condenser to protect the oil diffusion pump from any chloroform vapors which might possibly spill over from the first condenser. With this design much lower pressures could be reached and consequently, a favorable pressure differential between these PVP containing samples and the condensers was obtained, which allowed successful sublimation of the chloroform from these samples.

Results and Evaluation

Determination of the Adsorption Isotherms: Before the specific surface areas of the rapidly frozen-sublimation

dried samples could be determined using the nitrogen adsorption method, it had to be shown that the nitrogen adsorption isotherms were sigmoid shaped. Some question may have arisen as to the physical nature and adsorption characteristics of these substances if this were not done, since these products represent a new form of the steroids.

It was decided to determine the adsorption isotherms of samples of freeze-dried pregnenolone and cholesterol. For the isotherms, the samples prepared from 2½% solutions in chloroform were used. The isotherms are shown in Figure III, both having a sigmoid shape. Since both the pregnenolone and chloroform samples displayed S-shaped adsorption isotherms, the rapidly frozen-sublimation dried samples of Δ4-androstene-3,17-dione were presumed to follow the same pattern.

Effect of Concentration: Once the fact that these powders adsorbed nitrogen in a mono-molecular layer had been proven by the demonstration of sigmoid shaped isotherms, the determination of specific surface areas of these products was validated. With this analytical tool applicable, study of the process and its products was made possible.

The first thing to be studied was the effect of varying the concentration of steroid in the rapidly frozen solution. The results are tabulated in Tables II and III, and the curves obtained by plotting specific surface area

SUBLIMATION APPARATUS FOR
PVP CONTAINING SAMPLES

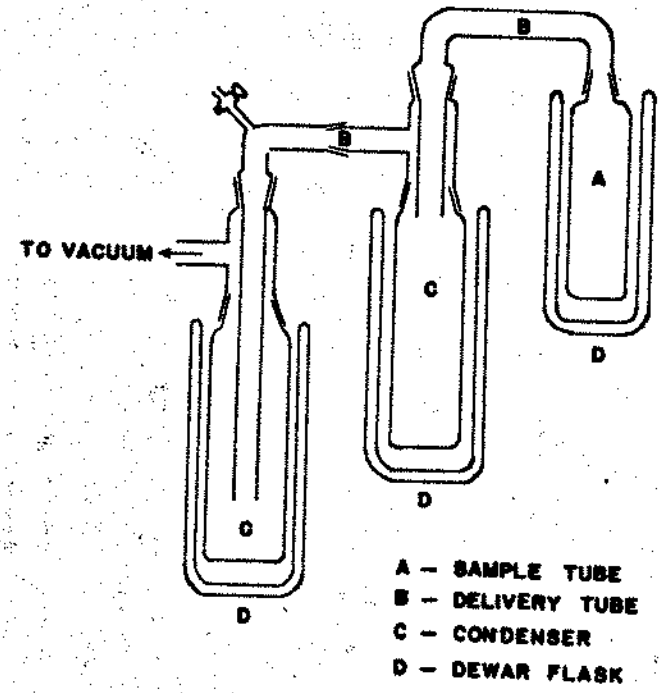

FIGURE 11

versus concentration of steroid in the frozen solution are shown in Figures IV and V.

From the curves it is seen that in both instances a maximum specific surface area is reached. In the case of cholesterol the maximum occurs when the concentration of cholesterol in the frozen solution is about 10%, whereas the maximum specific surface area of pregnenolone occurs when the concentration is approximately 5%. The specific surface area of the cholesterol powder is much higher than for pregnenolone, reaching an average of 21.75 square meters per gram at the maximum. In the case of pregnenolone, the maximum specific surface area is 5.74 square meters per gram. These values correspond to an average particle diameter of 0.256 microns and 0.893 microns, respectively. This large difference in particle size may be attributed to the intermolecular forces, which are also active in determining the solubility of the compounds. Pregnenolone is soluble in chloroform only to the extent of about 17% (47) while cholesterol is much more soluble.

That a critical concentration of steroid in the frozen solution exists at which the specific surface area of the powder obtained is maximal, is surprising. Normally, it would be expected that as the concentration of steroid in the frozen solution is decreased, the average particle size of the powdered steroid obtained would decrease. Even if the opposite were true and the specific surface area de-

ADSORBTION ISOTHERMS
OF RAPID FREEZE POWDERS

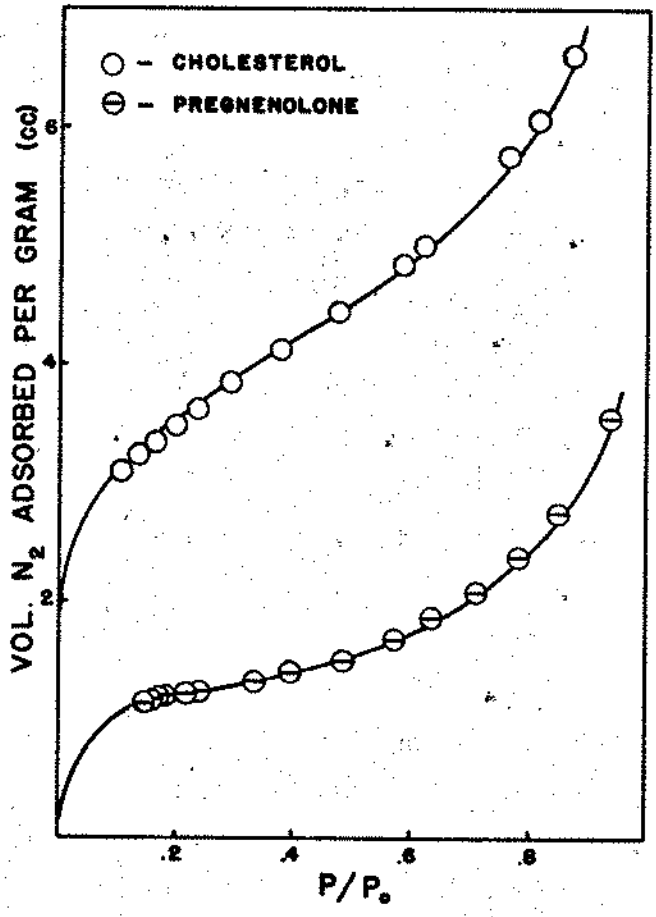

FIGURE III

TABLE II

PARTICLE SIZE OF CHOLESTEROL POWDER AS A FUNCTION OF CONCENTRATION IN FROZEN SOLUTION

Sample Rapidly frozen in Liquid Air, Maintained During Sublimation with an Acetone-Dry Ice Mixture.

Concentration in Frozen Solution	Sample Wt. (Gm_s)	V_m (cc)	Specific Surface Area (M^2/Gm_s)	Average Particle Diameter (microns)
1%	4.362	1.405	6.11	0.921
	4.360	1.395	6.07	
	4.885	1.355	5.89	
	3.289	1.411	6.14	
2.5%	3.290	2.970	12.92	0.434
	"	2.954	12.85	
	"	2.993	13.02	
	"	2.903	12.63	
5%	4.383	3.735	16.24	0.341
	4.802	3.746	16.30	
	"	3.779	16.44	
8%	2.215	4.447	19.34	0.289
	"	4.422	19.24	
8%	1.392	4.742	20.63	0.270
	"	4.729	20.57	
10%	4.618	4.917	21.39	0.258
	"	5.011	21.80	
10%	2.259	5.072	22.06	0.254
	"	5.005	21.77	
15%	2.468	4.022	17.50	0.319
	"	4.006	17.43	
15%	2.199	3.627	15.78	0.355
	"	3.626	15.77	
20%	3.472	3.362	14.62	0.382
	"	3.350	14.57	
30%	2.589	3.378	14.69	0.378
	"	3.434	14.94	
40%	2.290	0.645	2.81	1.978
	"	0.651	2.83	

TABLE III

PARTICLE SIZE OF PREGNENOLONE-POWDER AS A FUNCTION OF CONCENTRATION IN FROZEN SOLUTION

Sample Rapidly Frozen in Liquid Air, Maintained During Sublimation with an Acetone-Dry Ice Mixture.

Concentration in Frozen Solution	Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Average Particle Diameter (microns)
1%	4.640	0.587	2.55	1.988
	"	0.619	2.69	
	"	0.577	2.51	
2.5%	4.253	1.107	4.82	1.098
	"	1.042	4.53	
	"	1.073	4.67	
5%	2.527	1.334	5.80	0.893
	"	1.328	5.78	
	"	1.294	5.63	
10%	3.359	1.042	4.53	1.127
	"	1.054	4.58	
15%	3.858	0.581	2.53	2.068
	1.208	0.553	2.41	
	3.858	0.614	2.67	
	"	0.528	2.30	
	3.846	0.567	2.47	

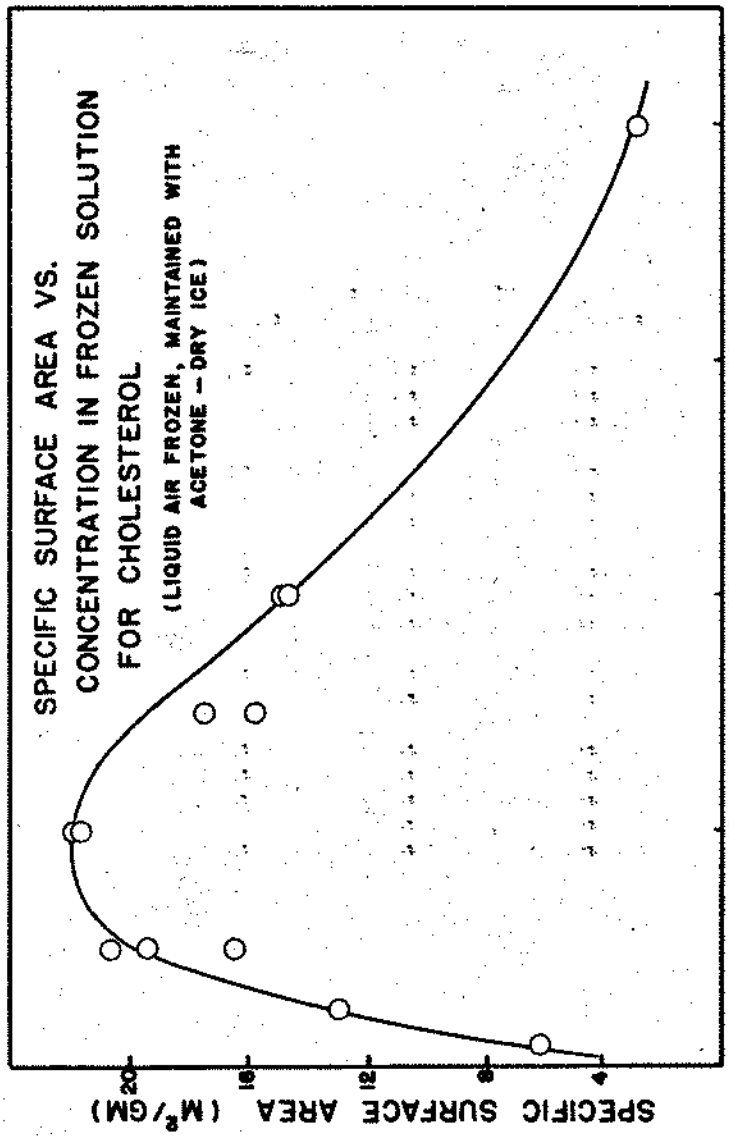
SPECIFIC SURFACE AREA VS.
CONCENTRATION IN FROZEN SOLUTION
FOR CHOLESTEROL
(LIQUID AIR FROZEN, MAINTAINED WITH
ACETONE — DRY ICE)
SPECIFIC SURFACE AREA (M²/GM)
4
8
12
16
20
10
20
30
40
CONCENTRATION (%)

FIGURE IV

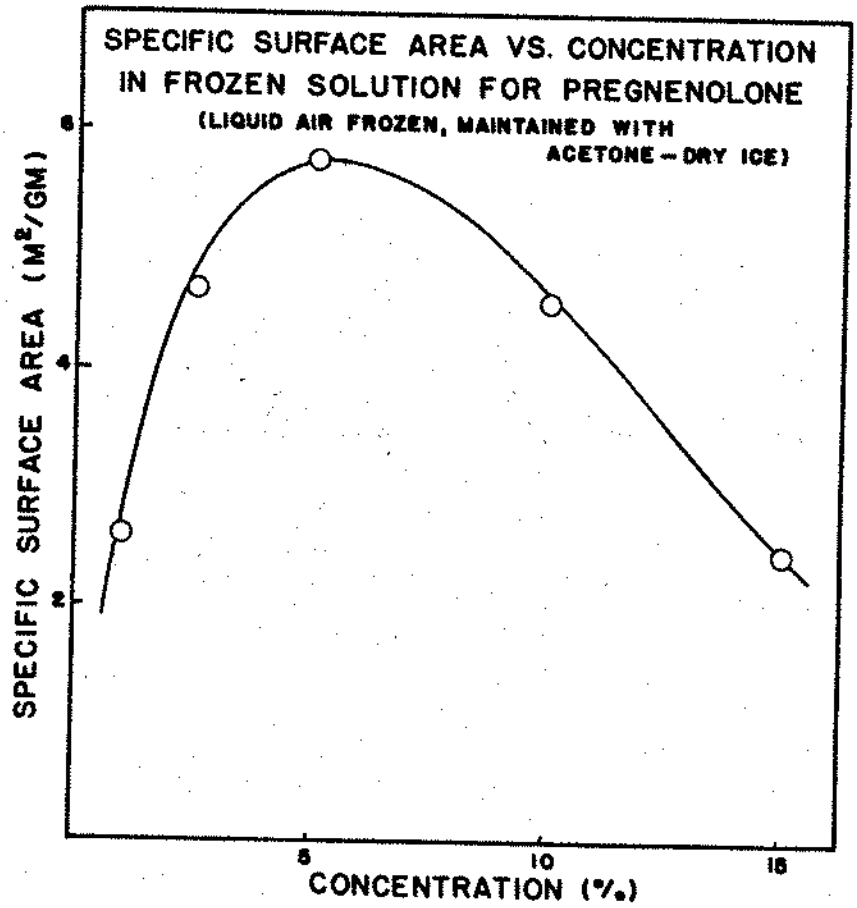
SPECIFIC SURFACE AREA VS. CONCENTRATION
IN FROZEN SOLUTION FOR PREGNENOLONE
(LIQUID AIR FROZEN, MAINTAINED WITH
ACETONE – DRY ICE)
SPECIFIC SURFACE AREA (M²/GM)
6
4
2
5
10
15
CONCENTRATION (%)

FIGURE V

creased with a decreased concentration of steroid in the frozen solution, an explanation based on known theory could be made. To explain the existence of this maximum in the specific surface area, however, it is necessary to resort to a postulation which will be proved or disproved by future work.

Probably the most plausible attack to the explanation for the maximum can be made by proposing that the steroid particles produced in the frozen solution do not have a true crystal form, but rather, have a gel type structure which gives rise to a honeycomb appearance. Such a structure would result when the solvent is removed from the frozen mass provided that the solute is distributed in a homogeneous manner throughout the mass. The pores in the particles would represent the spaces originally occupied by the solvent. The specific surface area of such a powder would be dependent not only on the size of the primary particles, but also on the degree of honeycombing in the particle.

As the concentration of steroid in the frozen solution is increased, the size of the primary particles produced by rapid freezing is expected to increase, but more important, the degree of honeycombing would decrease due to the deposition of the extra solute in the pores. This would account for the decrease in specific surface area found when solutions containing more than the

critical concentration of steroid are processed.

When concentrations of steroid less than the critical concentration are used in the frozen solution, the honeycomb structure of the particles produced may be very delicate. Such a situation would be magnified as the concentration of steroid in the frozen solution is progressively decreased. As the solvent is being removed from these frozen solutions, the finer portions of the steroid framework may collapse, thereby reducing the degree of honeycombing. This would account for the decrease in specific surface area found when powders are produced by processing dilute solutions.

Effect of Freezing Refrigerant: The next variation which was studied was that of changing the freezing medium to liquid nitrogen. Liquid nitrogen, which boils at -196° C., is about 10 degrees colder than liquid air, and it was desired to see whether or not this temperature difference was significant. The results of these studies are shown in Table IV, and Figure VI.

It is seen that the temperature difference between liquid nitrogen and liquid air is not sufficient to cause more rapid freezing and consequently, higher specific surface areas. Again a tendency toward a maximum exists in the vicinity of a 10% concentration of cholesterol in the frozen solution. For some reason, the powders

TABLE IV

PARTICLE SIZE OF CHOLESTEROL POWDER AS A FUNCTION OF FREEZING REFRIGERANT

Sample Rapidly Frozen in Liquid Nitrogen, Maintained During Sublimation with an Acetone-Dry Ice Mixture.

Concentration in Frozen Solution	Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Average Particle Diameter (microns)
5%	1.782	4.660	21.08	0.268
	"	4.770	20.55	
5%	1.367	4.657	20.26	0.271
	"	4.800	20.88	
10%	1.526	3.910	17.01	0.331
	"	3.837	16.69	
10%	1.569	4.575	19.90	0.278
	"	4.641	20.19	
	"	4.631	20.14	
10%	1.634	5.169	22.49	0.248
	"	5.241	22.80	
15%	2.045	3.963	17.24	0.329
	"	3.810	16.57	
15%	2.122	4.165	18.20	0.309
	"	4.118	17.91	
15%	2.693	3.677	16.86	0.333
	"	3.815	16.60	
15%	2.288	4.105	17.86	0.316
	"	4.011	17.45	
20%	2.746	3.414	14.85	0.373
	"	3.462	15.06	
20%	2.546	2.962	12.88	0.433
	"	2.957	12.86	
20%	2.482	3.035	13.20	0.419
	"	3.085	13.42	
30%	2.170	1.433	6.24	0.856
	"	1.556	6.77	

obtained by rapidly freezing solutions of this concentration in liquid nitrogen were not reproducible, their specific surface areas varying from about 16.85 square meters per gram to 22.66 square meters per gram. With samples obtained from the 15% and 20% solutions, a certain amount of variation was also noticed, but not nearly as pronounced as in the case of the 10% solutions.

Effect of Sample Temperature during Sublimation: The effect of the temperature at which the frozen sample was sublimed on the specific surface area was shown by replacing the acetone-dry ice cooling mixture usually used to keep the subliming sample frozen, with a cold air bath prepared by pre-chilling a Dewar flask with liquid air. The curve obtained by plotting the specific surface areas of the powders produced when the solution was frozen in liquid nitrogen against the concentration of cholesterol in the starting solution is shown in Figure VII, while the experimental results are tabulated in Table V.

It is apparent from the results that the specific surface area of the powder produced is diminished from that of a comparable sample when the subliming sample is maintained frozen with an acetone-dry ice mixture. This means that as the temperature of the subliming sample approaches the melting temperature of chloroform, growth of the steroid takes place. With the refrigeration

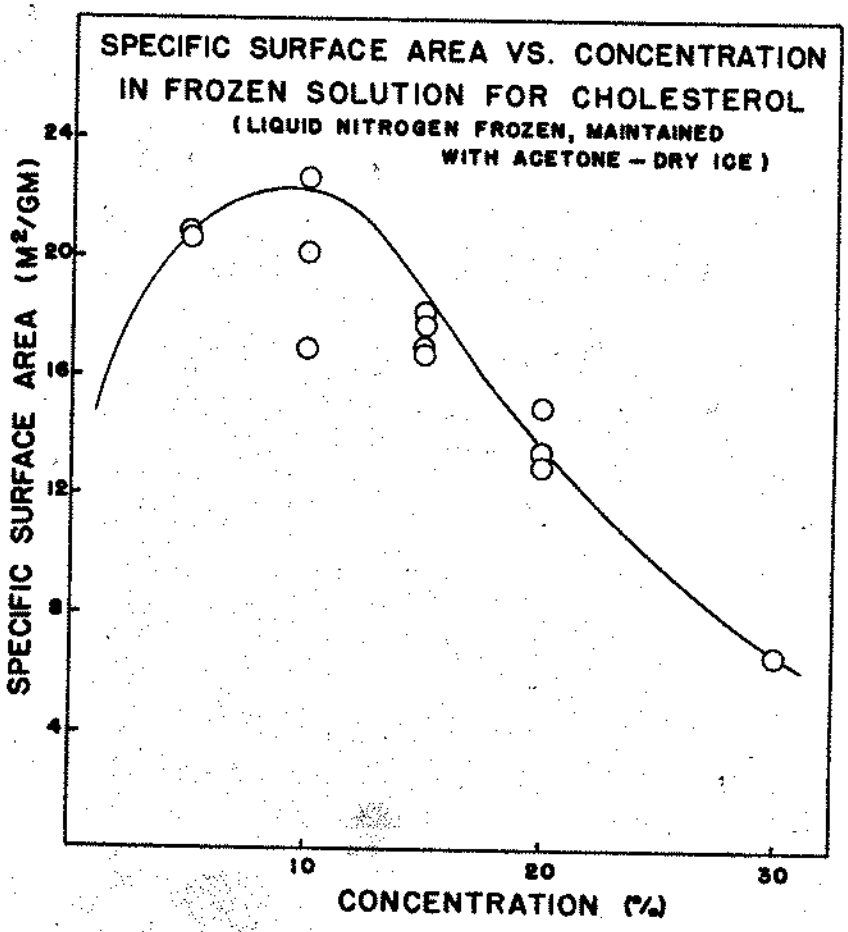
SPECIFIC SURFACE AREA VS. CONCENTRATION
IN FROZEN SOLUTION FOR CHOLESTEROL
(LIQUID NITROGEN FROZEN, MAINTAINED
WITH ACETONE – DRY ICE)
SPECIFIC SURFACE AREA (M²/GM)
24
20
16
12
8
4
10
20
30
CONCENTRATION (%)

FIGURE VI

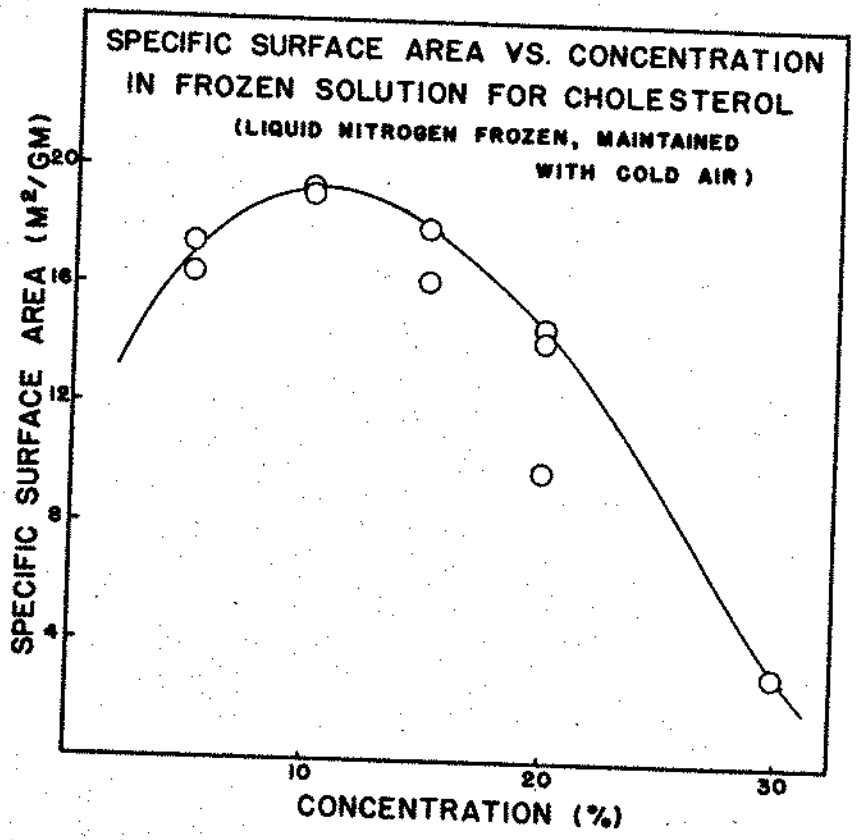
SPECIFIC SURFACE AREA VS. CONCENTRATION
IN FROZEN SOLUTION FOR CHOLESTEROL
(LIQUID NITROGEN FROZEN, MAINTAINED
WITH COLD AIR)
SPECIFIC SURFACE AREA (M²/GM)
20
16
12
8
4
10
20
30
CONCENTRATION (%)

FIGURE VII

TABLE V

PARTICLE SIZE OF CHOLESTEROL POWDER AS A FUNCTION OF SAMPLE TEMPERATURE DURING SUBLIMATION

Sample rapidly Frozen in Liquid Nitrogen, Maintained During Sublimation With a Cold Air Bath.

Concentration in Frozen Solution	Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm)	Average Particle Diameter (microns)
5%	1.775 "	3.827 3.739	16.65 16.26	0.338
5%	1.311 "	4.046 4.018	17.60 17.48	0.317
10%	2.316 "	4.395 4.461	19.12 19.41	0.289
10%	1.877 "	4.451 4.394	19.36 19.11	0.290
15%	2.020 "	4.138 4.149	18.00 18.00	0.310
15%	2.709 "	3.722 3.709	16.19 16.13	0.345
20%	2.869 "	2.535 2.491	11.03 10.84	0.509
20%	2.650 "	3.346 3.416	14.56 14.86	0.379
20%	2.660 "	3.267 3.300	14.21 14.36	0.390
30%	3.778 "	0.704 0.722	3.06 3.14	1.797

technique used in these studies, a question may arise as to whether or not the samples might have melted in these cases. If this did occur, it could have occurred only to a very slight degree since the visual, tactile and microscopic appearance of these powders was in no way different from that of the powders obtained when an acetone-dry ice mixture was used.

Two samples were also run in which the cholesterol solution was frozen in liquid air. From the data as shown in Table VI, it is seen that the specific surface area is reduced from that of corresponding samples in which an acetone-dry ice mixture was used as refrigerant for the subliming sample. This agrees with the results shown in the work with liquid nitrogen frozen samples comparing the effect of sample refrigerant.

Effect of Solvent: Exploratory work on the effect of the solvent for the steroid solution was also completed. Carbon tetrachloride was used in place of chloroform as solvent for the steroid. Both cholesterol and Δ4-androstene-3,17-dione were prepared, the cholesterol from a 10% solution and the Δ4-androstene-3,17-dione from a 5% solution in carbon tetrachloride. The cholesterol powder obtained showed a specific surface area of about 12.07 square meters per gram while the powdered Δ4-androstene-3,17-dione had a specific surface area of 5.94 square meters per gram. (See Table VII).

TABLE VI

PARTICLE SIZE OF CHOLESTEROL POWDER AS A FUNCTION OF SAMPLE TEMPERATURE DURING SUBLIMATION

Sample Rapidly Frozen in Liquid Air, Maintained During Sublimation with a Cold Air Bath.

Concentration in Frozen Solution	Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Average Particle Diameter (microns)
10%	1.832	3.758	16.32	0.346
	"	3.638	15.85	
20%	2.968	2.969	12.92	0.431
	"	2.977	12.95	

TABLE VII

PARTICLE SIZE OF VARIOUS POWDERS OBTAINED BY THE RAPID FREEZING METHOD.

All Samples Rapidly Frozen in Liquid Air, Maintained during Sublimation with an Acetone-Dry Ice Mixture.

Solvent	Solute	Conc.	Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Average Particle Diameter (microns)	Remarks
CCl_4	Chol.*	10%	1.780	2.764 2.805	12.02 12.20	0.466	
CCl_4	Chol.*	10%	1.672	2.762 2.768	12.01 12.04	0.463	
CCl_4	Andro.*	5%	2.477	1.385 1.363	6.02 5.93	0.843	
CCl_4	Andro.*	5%	2.046	1.345 1.356	5.85 5.94	0.855	
$CHCl_3$	Chol.*	10%	2.527	3.063 3.022	13.28 13.15	0.422	0.1% Tween mixture added.
$CHCl_3$	Chol.*	10%	2.350	2.096 2.089	9.12 9.09	0.612	0.1% Tween mixture added.
$CHCl_3$	Chol.*	10%	2.242	4.008 4.044	17.45 17.59	0.315	0.1% absolute ethanol added.
$CHCl_3$	Chol.*	10%	1.641	4.441 4.547	19.32 19.78	0.285	0.1% absolute ethanol added.

TABLE VII (cont.)

Solvent	Solute	Conc.	Sample Wt. ($Gm._s$)	V_m (cc)	Specific Surface Area ($M^2/Gm._s$)	Average Particle Diameter (microns)	Remarks
$CHCl_3$	Chol.*	10%	1.544	1.994 1.976	8.67 8.60	0.648	Sprayed into liquid air.
$CHCl_3$	Chol.*	10%	1.418	4.349 4.449	18.92 19.35	0.291	Sprayed into liquid air.
$CHCl_3$	Chol.*	10%	0.696	2.896 2.989	12.60 13.00	0.438	Sprayed into liquid air.
$CHCl_3$	Chol.*	10%	3.491	0.784	3.18	1.768	10% absolute methanol added.
$CHCl_3$	Chol.*	10%	2.845	0.688 0.678	2.99 2.95	1.876	10% absolute methanol added.

*Cholesterol
° Δ4-Androstene-3,17-dione

Since the specific surface area of a similarly treated 10% solution of cholesterol in chloroform is about 21.75 square meters per gram, the difference in specific surface areas of the two powders can only be explained on the basis of some action of the solvent. If the solvent were inert in the process, the specific surface areas would be expected to be identical. That the solvent should play an active part in determining the particle size of the steroid powder is to be expected since this is a precipitation procedure.

The mechanism whereby the solvent exerts its influence on the average particle size of the steroid powder produced may be twofold. Firstly, the interaction between solvent and solute may be of importance, and secondly, the type of crystal formed by the solvent on freezing may have an effect. In the first instance, it seems logical to assume that the solvent which interacts with the solute to a greater extent will be more capable of preventing the solute from agglomerating during the freezing process. If the interaction is greater, the solubility of the steroid will be greater; therefore, a greater degree of cooling will be required to produce the degree of supersaturation necessary to initiate crystallization. This means that the critical period between the onset of crystallization and the production of a sufficient viscosity to halt crystallization will be shorter, resulting

in a finer particle size distribution of steroid in the frozen mass.

The nature of the solvent crystal is also important in the production of finely subdivided powders. If the solvent crystal has strong intermolecular forces, such that the solute molecules are squeezed out of the crystal rather than entrapped, the solute molecules will be free to aggregate and form larger particles. The ideal solvent would be one which would form a solid solution with the steroid when frozen. In such a case, the distribution of steroid throughout the frozen mass would be completely homogeneous, provided that the freezing was sufficiently rapid. With solvents such as chloroform and carbon tetrachloride, a solid solution situation is approached, but with a solvent such as water, it is very possible that the steroid would be excluded from the solvent crystal.

Effect of Rate of Freezing: An attempt to determine the effect of rate of freezing was made in which a 10% solution of cholesterol in chloroform was sprayed into the liquid air. By introducing the solution in the form of fine droplets, it was reasoned that the freezing should occur more rapidly, thus producing a finer particle size of steroid in the frozen mass.

Considerable difficulty was encountered in the

removal of solvent from these samples. The appearance of the frozen solution, frozen in the form of very fine droplets was that of a slushy mass. As a result, the mass was very compact in the sample tube during the drying procedure, which made removal of the last portions of solvent very difficult. This is explained by the fact that the vapors were retarded in their escape by the crust of previously dried powder.

The specific surface areas of the samples of cholesterol powder prepared in this way were 8.64 square meters per gram, 19.14 square meters per gram and 18.80 square meters per gram (see Table VII). In view of the fact that removal of solvent was difficult, it would seem plausible to explain this deviation by assuming that minute portions of the sample melted during the drying procedure. On the other hand, macroscopic and microscopic observation of the samples failed to show any difference between these samples and the others. If melting had occurred, it would be expected that definite crystalline shapes would be observed.

The alternate explanation for this large difference in specific surface areas between samples is that the larger particles of steroid in the frozen mass grew by attracting the smaller aggregates of steroid during the sublimation of the solvent. Perhaps on freezing these solutions as fine droplets, the freezing is so rapid

that a considerable quantity of steroid is distributed throughout the frozen solution as molecular aggregates which have an appreciable vapor pressure. As the solvent sublimes these aggregates might vaporize and be deposited on the larger particles of steroid. An indication that this may be the true explanation is the fact that only about 50% of the original steroid is recovered as powder in this process, the remainder being carried over with the solvent vapors to the condenser. More work in this direction must be performed before any conclusions may be drawn.

Effect of Additives: The final studies with the rapid freezing-sublimation drying technique concerned themselves with the effect of adding foreign substances to the solution before freezing. The additives were of two types, liquid and solid.

With the liquid additives, the specific surface areas of the resultant powders were measured since the liquid was either removed on sublimation drying, or added only in trace amounts. In this series, absolute ethanol, absolute methanol and the mixture of Tweens previously mentioned were added to the initial solution.

From the results (see Table VII) it is seen that these agents reduce the specific surface area. In the case of the Tween mixture the observed decrease is

probably due to the occluding of some of the pores in the crystal by the additive and the aggregating of the primary particles which are coated with a film of the Tween mixture. With ethanol and methanol the results can best be explained by postulating a growth of the steroid particles through the alcohol. Both methanol and ethanol freeze at temperatures below that of an acetone-dry ice mixture and therefore, they exist in a liquid form in the sample before removal by evaporation under reduced pressure. The extreme diminution of specific surface area with the use of a large quantity of methanol tends to bear out this contention.

With the solid additives, it was intended to find a protective agent which would trap the particles of steroid in its framework. It was thought that any aggregation of the particles during sublimation drying would be eliminated and that possibly the solubility of the steroid could be increased, because the particles would be extremely small. Because of the large quantity of additive added, specific surface area measurements would be meaningless as an index to the particle size of the steroid. Analysis, therefore, consisted of preparing aqueous suspensions of the powders obtained, and making visual observations.

The results when caffeine was used as the water soluble protective agent were not very promising. The

powders resulting from the process were readily wetted, but as the caffeine went into solution, the cholesterol particles started to agglomerate. The samples in which PVP was used gave better results. Again the powders were wetted, although the PVP showed a tendency to slump when wetted with water. After the PVP had dissolved, a finely distributed suspensoid resulted, but here too, a tendency for the suspended steroid to coagulate was observed. To remedy this, the mixture of Tweens was added in small quantities to the suspensions. The reduction of surface tension produced acted to give these suspensions stability. On dilution of the more concentrated suspensions dispersions of almost colloidal characteristics resulted. The best results were attained with a ratio of three to five parts of PVP to one part of cholesterol in the frozen solution. Addition of a small amount of the Tween mixture to the solution before freezing produced a powder which was very readily wetted by water.

The investigations in this direction were only exploratory, and from them it can be seen that many interesting possibilities exist for the application and improvement of this technique.

SPRAY DRYING INVESTIGATIONS

The adaptation of the spray drying procedure to the formation of submicron particles was made in an effort to

obtain a limited comparison of the value of this procedure with that of rapid freezing and sublimation drying. Because of the limited facilities available in these laboratories, this method was not expected to give comparable results. The primary reason for this was the lack of facilities to collect those particles with a diameter less than one micron produced by spray drying. In fact this very problem tends to rule out entirely the consideration of this process as a method to achieve the goal of this investigation.

Two samples of cholesterol were prepared using a Bowen Laboratory Model spray drier. The first sample was prepared by spray drying a 5% solution of cholesterol in chloroform, the second by spray drying a 1% solution of cholesterol in chloroform. Both samples were preserved for the determination of the specific surface area.

Since spray dried products had already been analyzed for specific surface area by the nitrogen adsorption method, the adsorption isotherm of spray dried cholesterol was not determined. (52) The powdered cholesterol obtained by spray drying the 5% solution in chloroform was found to have a specific surface area of 1.30 square meters per gram and the cholesterol sample produced by spray drying the 1% solution had a specific surface area of 1.08 square meters per gram. These surface areas correspond to an average particle diameter of 4.64 microns.

It would be expected that as the concentrations of solute in the spray dried solution decreased, the average particle size of the powder obtained would decrease, provided that the controlling factors, such as disc speed and feed rate, were kept the same. In this work it was found that the particle sizes obtained were identical with a concentration of 5% or 1% in the spray dried solution. This can be related to the fact that the cyclone collector used in this work was not capable of collecting the smaller particles produced. Consequently, the experimental results are only a measure of the size of the particles classified by the cyclone collector employed and can not be taken as an indication of the size of the particles produced by the spray drying procedure. The finer spray dried particles of steroid were not collected, but passed out of the apparatus with the exhaust gases.

PART II. INFLUENCE OF THERMAL AGING ON THE SPECIFIC SURFACE AREA.

Theoretical

Because of the manner in which these powdered steroids are produced, it might be expected that they exist in a relatively unstable form. One indication that this is the case is the extremely high specific surface area associated with these powders. The large surface area indicates that the surface energy on the steroid in this form is very high, much higher than it is when the steroid is in a thermodynamically stable state.

If these powders do represent a thermodynamically unstable form of the steroids, external forces may act to alter the energy relationships, enabling the steroid powder to approach more closely to a thermodynamically stable state. The simplest form in which an outside force can be applied to the powder is in the form of thermal energy, and if the foregoing consideration is true, an alteration in the physical nature of the steroid powders will occur.

Experimental

The effect of the influence of thermal aging was studied with two different samples of cholesterol prepared by the rapid freezing-sublimation drying process. The first sample was allowed to stand at room temperature

while the second sample was aged at elevated temperatures.

In the first study a sample of cholesterol prepared from a 10% solution in chloroform using an acetone-dry ice mixture to maintain the subliming sample was used. The specific surface area was determined initially and then the sample was allowed to stand at room temperature for a period of about five months. The specific surface area of the sample was re-determined at approximately one month intervals.

For the second study a large quantity of rapidly frozen-sublimation dried cholesterol was prepared from a 10% solution in chloroform using a cold air bath to maintain the drying sample. To prepare a suitable quantity of this material, several runs were required. The individual batches were intimately mixed and the specific surface area of the total sample was determined.

From this large sample, smaller portions were taken and treated in a vacuum oven at temperatures of 45° C., 55° C. and 64° C. for specified periods of time. After each heating, the specific surface area of the powder was determined. Duplicate samples were similarly treated to obtain a check on the results.

Results and Evaluation

That the specific surface area of the freeze-dried cholesterol decreases on standing at room temper-

ature is shown by the results tabulated in Table VIII, and the curve obtained when the specific surface area of the powder is plotted against the time of standing (Figure VIII). The rate of decrease of surface area is seen to fall off as the length of time increases.

More revealing data resulted from the studies made at increased temperatures. The results of these studies are shown in Table IX, and Figures IX, X and XI, in which surface area has been plotted as a function of the time of heating for temperatures of 45° C., 55° C. and 64° C. respectively.

Analysis of the results at these three temperatures shows that the decrease in specific surface area is very temperature dependent. In other words, a high temperature coefficient is to be expected in the process.

Since the rate of diminution of the specific surface area is dependent upon the temperature, it may be expected to conform approximately to the Arrhenius equation which states:

$$\frac{d \ln r}{dT} = \frac{\Delta H}{RT^2}$$

where r is the rate of decrease in specific surface area at absolute temperature, T, ΔH is the heat of activation and R is the gas constant. Integration of this expression, assuming ΔH to be constant, gives:

$$\log r = -\frac{\Delta H}{2.303\ R} \cdot \frac{1}{T} + C$$

TABLE VIII

ROOM TEMPERATURE AGING OF CHOLESTEROL POWDER PREPARED BY THE RAPID FREEZE METHOD

Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (M^2/Gm.)	Date of Determination
4.618 "	4.917 5.011	21.39 21.80	12/17/51 (initial)
1.483 1.605	4.605 4.677	20.03 20.34	2/ 5/52
1.573 "	4.398 4.340	19.11 18.88	3/7/52
1.608 "	4.541 4.494	19.78 19.65	4/9/52
1.644 1.682	4.478 4.413	19.48 19.20	5/ 9/52

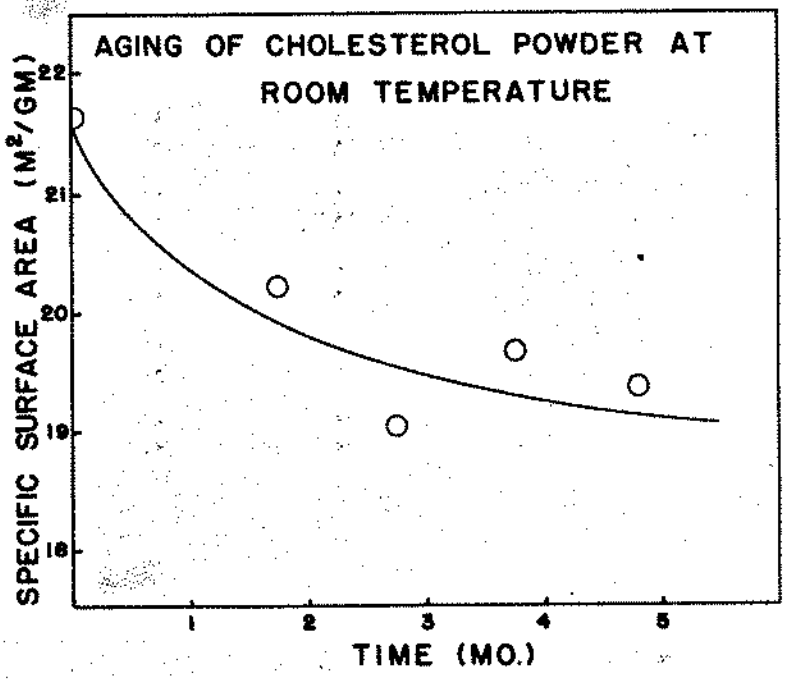
AGING OF CHOLESTEROL POWDER AT
ROOM TEMPERATURE
SPECIFIC SURFACE AREA (M^2/GM)
22
21
20
19
18
1
2
3
4
5
TIME (MO.)

FIGURE VIII

TABLE IX

ELEVATED TEMPERATURE AGING OF CHOLESTEROL POWDER PREPARED BY THE RAPID FREEZE METHOD

Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Treatment
1.532	3.638	15.83	Original Sample.
"	3.755	16.32	
1.522	3.700	16.10	Heated one hour at 45° C.
"	3.686	16.03	
1.485	3.658	15.91	Heated two hours at 45° C.
"	3.666	15.95	
1.522	3.463	16.06	Heated four hours at 45° C.
"	3.579	15.57	
1.537	3.582	15.58	Heated eight hours at 45° C.
"	3.558	15.48	
1.504	3.398	14.78	Heated 16 hours at 45° C.
"	3.473	15.11	
1.497	3.397	14.78	Heated 32 hours at 45° C.
"	3.421	14.88	
1.592	3.558	15.48	Heated 32 hours at 45° C.
"	3.499	15.22	
1.483	2.993	13.02	Heated 64 hours at 45° C.
"	3.091	13.45	
1.571	3.168	13.78	Heated 64 hours at 45° C.
"	3.218	14.00	
1.483	3.047	13.25	Heated 93 hours at 45° C.
"	2.962	12.88	
1.571	3.186	13.86	Heated 93 hours at 45° C.
"	3.180	13.83	
1.664	3.228	14.04	Heated 2 hours at 55° C.
"	3.280	14.27	
1.668	3.336	14.51	Heated 2 hours at 55° C.
"	3.416	14.86	
1.627	2.916	12.68	Heated 4 hours at 55° C.
"	2.952	12.84	

TABLE IX (cont.)

Sample Wt. (Gm.)	V_m (cc)	Specific Surface Area (m^2/Gm.)	Treatment
1.667	3.176	13.88	Heated 4 hours at 55° C.
"	3.275	14.25	
1.568	3.118	13.54	Heated 4 hours at 55° C.
"	3.190	13.88	
1.651	2.874	12.50	Heated 8 hours at 55° C.
"	2.946	12.82	
1.661	3.159	13.74	Heated 8 hours at 55° C.
"	3.105	13.51	
1.662	2.877	12.51	Heated 16 hours at 55° C.
"	2.942	12.80	
1.681	3.030	13.18	Heated 16 hours at 55° C.
"	3.068	13.43	
1.532	2.672	12.49	Heated 1 hour at 64° C.
1.552	2.745	11.94	Heated 1 hour at 64° C.
1.532	2.820	12.25	Heated 2 hours at 64° C.
1.552	2.790	12.13	Heated 2 hours at 64° C.
1.532	2.650	11.53	Heated 4 hours at 64° C.
1.552	2.725	11.84	Heated 4 hours at 64° C.
1.552	2.480	10.78	Heated 8 hours at 64° C.
1.552	2.350	10.21	Heated 8 hours at 64° C.

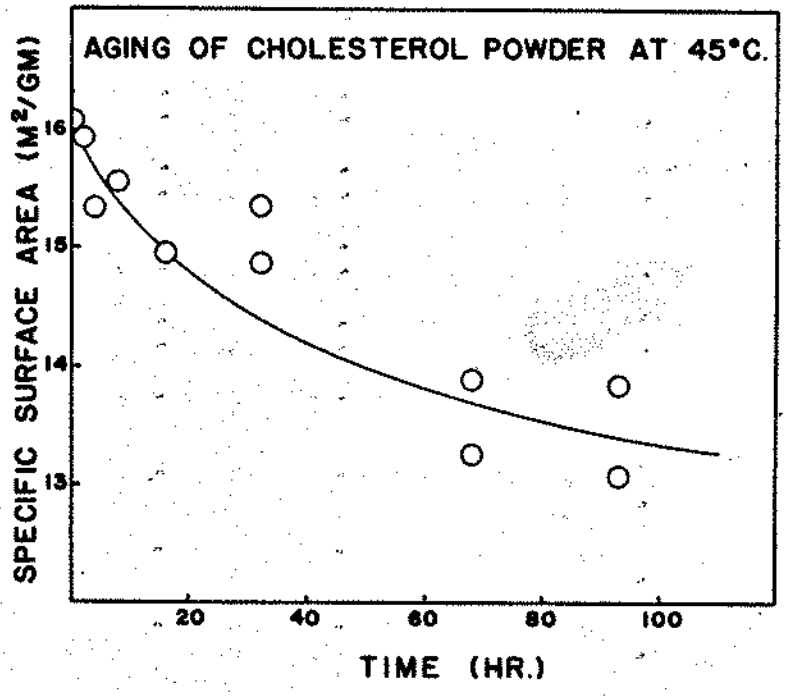
AGING OF CHOLESTEROL POWDER AT 45°C.
SPECIFIC SURFACE AREA (M^2/GM)
16
15
14
13
20
40
60
80
100
TIME (HR.)

FIGURE IX

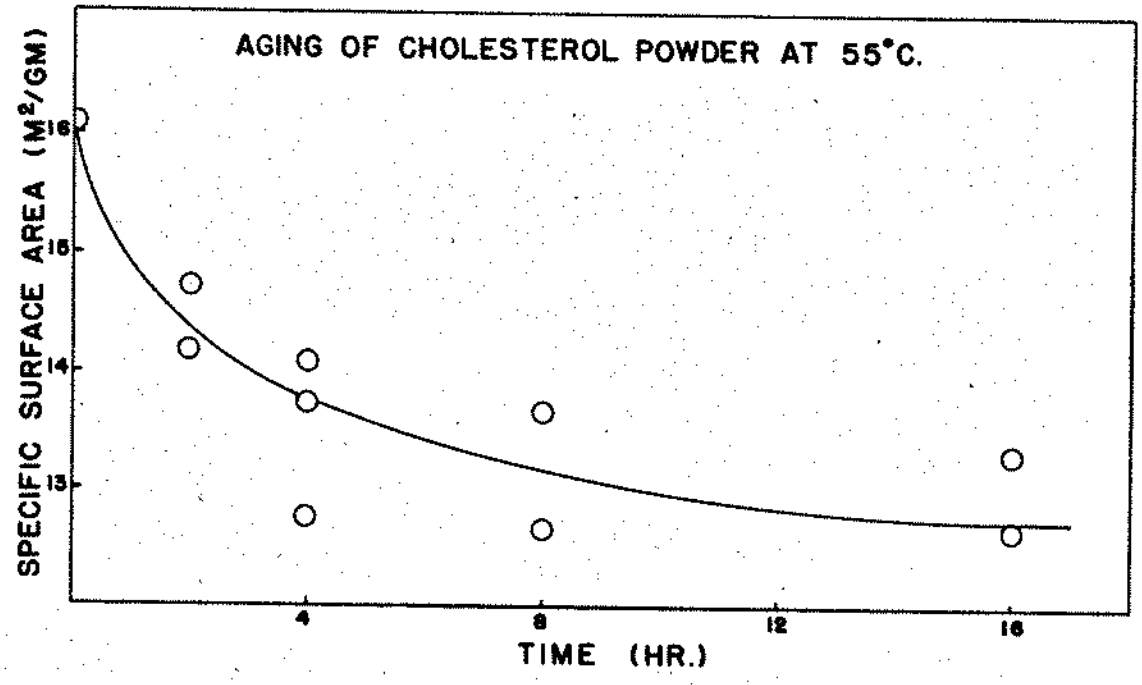
AGING OF CHOLESTEROL POWDER AT 55°C.
SPECIFIC SURFACE AREA (M²/GM)
1.6
1.5
1.4
1.3
4
8
12
16
TIME (HR.)

FIGURE X

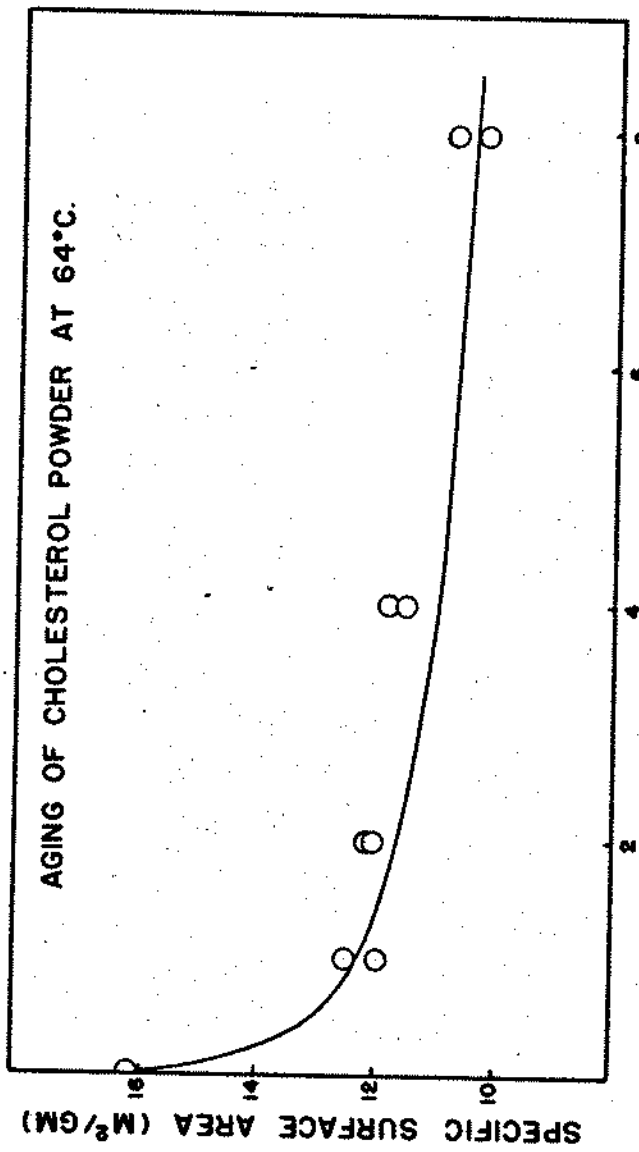
AGING OF CHOLESTEROL POWDER AT 64°C.
SPECIFIC SURFACE AREA (M²/GM)
TIME (HR.)
0
2
4
6
8
10
12
14
16

FIGURE XI

From this equation it is seen that a plot of log r versus 1/T will give a straight line whose slope equals $= \frac{\Delta H}{2.303\ R}$.

The reduction in specific surface area per unit time, at constant temperature, measures the rate of this process. Since all the heated samples were taken from the same source, the time required for the specific surface area to be reduced to a desired value at the three temperatures studied is a direct measure of the rate of decrease. For example, the time required for the specific surface area to reach a value of 14 square meters per gram can be determined from Figures IX, X and XI for each temperature, and this is equivalent to the rate of the process at these temperatures. Consequently, the logarithm of the time required to reduce the specific surface area to the desired level (log t) can be substituted for log r in the plot of the equation.

When the desired level for the specific surface area is set at 14 square meters per gram, the time required to reach this value is 15 minutes at 64° C., 190 minutes at 55° C. and 5000 minutes at 45° C. The logarithm of these times has been plotted against the reciprocal of the absolute temperature in Figure XII to show that the straight line relationship is followed. From the slope of the line obtained the value of ΔH has been calculated and found to be approximately 80 kilocalories per mole.

The high energy of activation for the process

ARRHENIUS PLOT OF ELEVATED TEMPERATURE AGING OF CHOLESTEROL POWDER PREPARED BY RAPID FREEZING

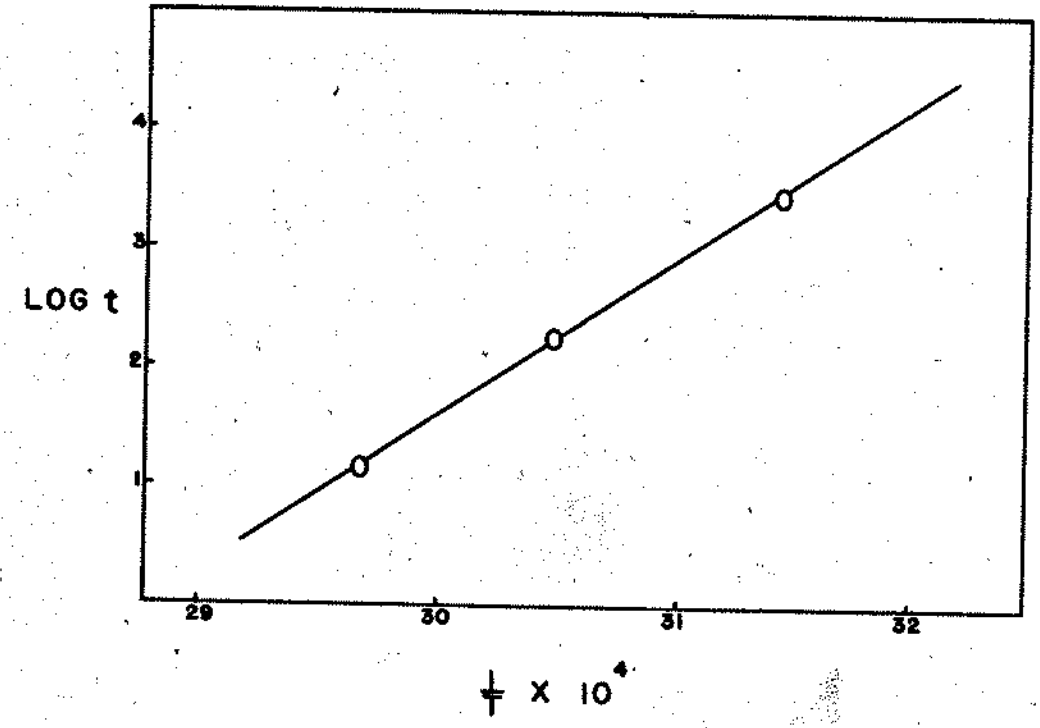

FIGURE XII

tends to rule out the possibility that the observed decrease in specific surface area is the result of a vaporization process wherein the molecules of steroid in the smaller aggregates, or those molecules held with weak intermolecular bondings (unstable crystalline positions) vaporize, only to condense on the larger particles in more stable orientations. Rather, the high energy requirement for the process would seem to point to a melting, a solid phase transition or some other cooperative phenomenon concerning the active individual particles. Such cooperative phenomena would exhibit the very high temperature coefficient noted in the present case.

In order to postulate a logical mechanism by which the observed changes might occur, it is necessary to be familiar with the theories of melting. Because the melting phenomenon is a cooperative phenomenon, an understanding of it will serve as a foundation for the explanation of the results obtained on thermal aging of the freeze-dried powders. For this reason, a brief consideration of the phenomenon or melting is included.

Most of the treatment of the melting phenomenon has been initiated from the standpoint of thermodynamics, or statistical mechanics. To discuss these theoretical approaches, however, would require a more detailed dissertation than is warranted by this research. Therefore, this discussion will be limited to a brief, qualitative description.

The classic theory of melting is that proposed by Lindemann. (48) According to this theory, as the temperature is raised, the amplitude of vibration of the atoms or molecules in the crystal lattice increases. When the amplitude of vibration becomes equal to the average distance between the atoms or molecules, collisions occur with the result that energy is shared and destruction of the crystal (melting) results. More recently melting has been related to the gradual increase in volume of the crystal which develops on heating. (49,50) As the volume increases, the regularity characterizing the initial crystal decreases until finally an amorphous body is produced. If the volume of the crystal is increased by reducing the pressure, holding the temperature constant, the amorphous body produced may be solid or liquid, depending upon the temperature. If the change of phase is induced by increasing the temperature at a constant pressure, a liquid is produced.

The thermodynamic quantity with which melting is most intimately related is the Gibbs free energy which is dependent upon the internal energy and the entropy of the system. A plot of the Gibbs free energy as a function of temperature for a simplified model of solid and liquid is shown in Figure XIII, the horizontal line representing the solid phase and the sloping line the liquid phase. At the point of intersection of the two curves, melting occurs. The phase which is more stable is the one in which the

IDEALIZED PLOT OF THE GIBBS FREE ENERGY OF A SUBSTANCE

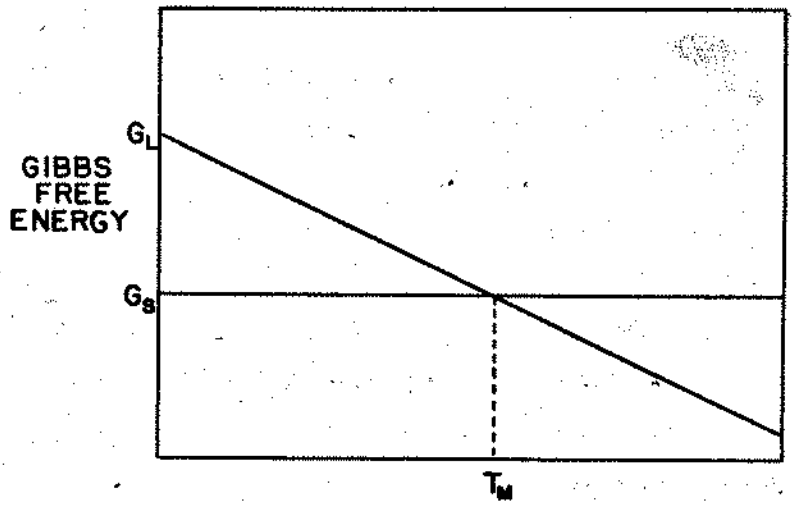

FIGURE XIII

Gibbs free energy is lesser. From the graph it can be seen that at temperatures below the melting point the solid phase has the lower free energy due to its lower internal energy. At temperatures above the melting point the liquid phase exists because at these temperatures, the increased entropy of the liquid is sufficient to counteract the greater internal energy. (51)

The dependence of the melting range upon the particle size of the crystal has been shown by Slater in a theoretical consideration of melting. (51) He considered an extremely small crystal, containing only a few hundred atoms, and showed that the temperature range in which the change of phase occurred might be of the order of a fraction of a per cent of the melting temperature. This would correspond roughly to a temperature range of a degree or so.

With this background, further consideration of the phenomenon observed in this research can be undertaken.

It is possible that in the formation of these powders by the rapid freezing-sublimation drying method, the steroid precipitates in an amorphous form in which the orientation of the molecules of steroid leaves them in relatively unstable positions. The energy relationships in such a situation may be presented as in Figure XIV where the energy levels of the stable molecules at A and the unstable molecules at B are represented graphically. As thermal energy is supplied to the system, the energy

ENERGY OF A MOLECULE AS A FUNCTION OF ITS ORIENTATION IN THE AGGREGATE

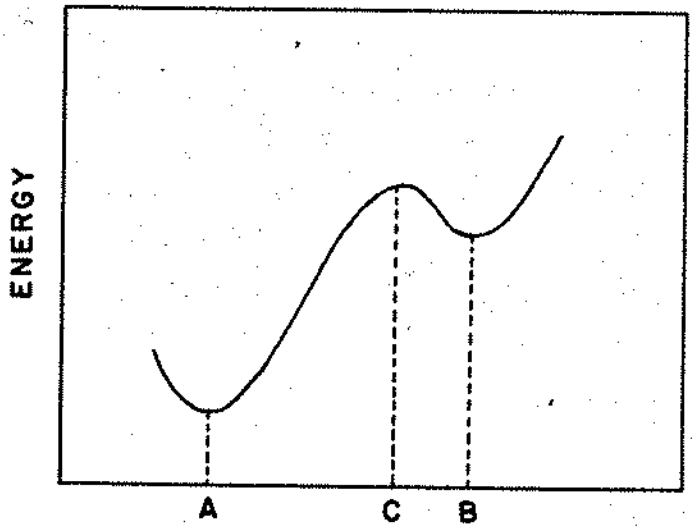

FIGURE XIV

level of the molecules at position B is increased, and if sufficient energy is supplied, these molecules attain the level of the critical position shown by point C. These molecules are then capable of either returning to position B or moving to the more stable energy level shown at position A. On the other hand, only rarely will a molecule at A absorb sufficient energy to transfer to position B.

The physical interpretation of this hypothetical situation is that the molecules at B correspond to those forming peaks and ridges on the amorphous body while the molecules at A correspond to those fitted into the more orderly crystalline lattice. As the peaks and ridges become levelled off (due to the shifting of the molecules which comprise them), the surface area of the particle decreases.

A mechanism such as is postulated to explain the decrease in surface area approached being a phase transition phenomenon. In a phase transition the energy level of all the molecules of the system is raised to such an extent that they are capable of passing over one another freely, reaching a final stability either as a new crystal form or as a liquid. In this case, only those molecules in unstable positions become capable of movement. These molecules then seek stability in the crystal lattice.

It must be borne in mind that the interpretation of the results in this report is based on limited experi-

mental findings. Before any definite statements can be made concerning the observed phenomenon, far more experimental work will be required.

CONCLUSIONS

This research has been largely qualitative in aspect since it has concerned itself with a technique with which little previous work has been done. From this type of study only broad conclusions can be drawn, but much of the value in this type of work lies in the fact that the areas for future study are brought sharply into focus. From this particular work the following conclusions can be drawn:

1. Steroids can be prepared in the form of submicron particles by rapidly freezing solutions of them and removing the solvent by sublimation from the frozen mass. Whether this is a practical method or merely a laboratory curiosity will be found by future examination of the process.

2. Laboratory scale sublimation units for removing the frozen solvent as a vapor have been designed, and the rapid freezing-sublimation drying process has been described. The method as described and the sublimation units diagrammed herein are not claimed to be highly efficient.

3. The average particle size produced by the rapid freezing-sublimation drying method is dependent upon the concentration of steroid in the solution which is frozen. A critical concentration is found at which the average particle size has its least value. This critical concen-

tration varies for each steroid processed by this method.

4. The average size of the particles produced by this method is dependent upon the temperature at which the frozen solution is maintained during the sublimation of solvent. As the temperature of drying is increased, the particles in the frozen mass become more capable of growing in size during the removal of the solvent.

5. The solvent employed for the steroid plays a role in affecting the average size of the particles obtained by this method.

6. No significant difference in the average particle size of the resulting steroid powder is noted when the solution is rapidly frozen in liquid air as compared to liquid nitrogen.

7. The addition of foreign substances to the solution of steroid before freezing affects the average particle size of the powder obtained. When polyvinylpyrrolidone is added to the solution in a ratio of approximately four parts PVP to one part of steroid, the resulting steroid particles can be prepared in the form of a dilute, colloidal suspension with the aid of a small amount of a surface active agent.

8. The steroid powders prepared by this method in the absence of protective agents are unstable from the standpoint of retaining their original average particle size. The rate at which the specific surface area of

these powders decreases is highly dependent upon the temperature, and follows the relationship described by the Arrhenius equation.

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Approved Dale E. Hurster

Date July 2, 1952

The Preparation of Steroids as Submicron Particles

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When aqueous suspensions of medicinal agents are administered parenterally, there exists a possibility that the solids introduced into the tissues will be irritating. The ability to irritate the tissue cells is a function of both the size of the particle and its physical properties. If the size of the suspended particles is reduced sufficiently, the irritation will be minimized and the difficulties associated with the irritation will be alleviated.

The steroids represent a class of compounds which are often administered parenterally in the form of aqueous suspensions, but because of their physical nature, it is very difficult to produce them in a fine state of subdivision. Many techniques have been tried in an effort to produce these compounds as fine powders, but to the present time none have proved completely satisfactory.

In this investigation, a new approach to the solution of the problem has been used. Solutions of the steroids were rapidly frozen by dropping them into liquid

air or liquid nitrogen. The solvent was then removed as a vapor from the frozen solution by placing the frozen mass in a closed system and reducing the pressure. When the solvent sublimed, the steroid was left behind in the form of a finely subdivided powder, the size of the primary particles depending upon several factors.

The most important requirement for the production of fine particles of steroid by this method is that the freezing of the solution be sufficiently rapid. By rapidly freezing the solution, the critical period during which crystals of steroid might form, is minimized. This critical period exists between the instant at which nucleation of the solution starts and the point at which the viscosity of the solution is increased to such an extent by the cooling that crystal growth is negligible.

In order to investigate the possibilities and limitations of the process employed in this work, a number of factors were studied to determine their influence on the final product. The investigations were limited to exploratory studies from which only generalized conclusions could be drawn because the scope of these studies was so broad. Since the objective of the work was to produce finely subdivided powders, it was decided to use the specific surface area as the criterion for judging the effect of the variations in the procedure which were investigated. The specific surface area is directly related to the particle

fineness and can readily be determined by the nitrogen adsorption method.

The first study concerned itself with the effect of varying the concentration of steroid in the solution which was frozen. It was found that a critical concentration exists at which the specific surface area has a maximum value. For solutions of cholesterol in chloroform, the maximum occurred when the concentration of cholesterol in the solution was approximately 10 per cent. The specific surface area of the powders obtained by processing such a solution was about 21.75 square meters per gram, which corresponds to an average particle diameter of 0.256 microns.

It was found that the temperature of the sample during the sublimation of the solvent is also active in regulating the specific surface area of the powder obtained. The closer the sample temperature approached the melting point of the solvent, the smaller was the specific surface area of the powder produced. Other factors which were shown to have an effect on the average particle size of the resulting powders were the solvent for the steroid, the rate of freezing of the solution and the addition of foreign substances to the solution.

Of particular interest is the observation that PVP (polyvinylpyrrolidone), when used as an additive, apparently enmeshes the steroid particles in its molecular

framework during the freezing process. As a result, very fine particles of steroid are produced. It is possible to form dilute colloidal suspensions of these particles with the aid of a surface active agent.

In addition to the investigation of the rapid freezing-sublimation drying process, the physical nature of the particles produced by this method was also studied. Samples of cholesterol powder produced by this method were subjected to thermal influences and the resulting change in specific surface area was determined. It was found that even at room temperature the specific surface area of the cholesterol powders decreased.

The reduction in specific surface area showed a very high temperature coefficient, indicating that the powders produced by this method are very sensitive to thermal influences. By measuring the rate of decrease in specific surface area at three temperatures, it was possible to show that the phenomenon conforms approximately to the Arrhenius equation, and that it has an activation energy in the neighborhood of 60 kcal. per mole.

The magnitude of the activation energy leads to the conclusion that the process by which the specific surface area is diminished is a cooperative phenomenon similar to melting or a solid phase transition. It is entirely possible that the method of preparing these

powders leads to the production of the steroid in a thermodynamically unstable state. Consequently, when energy is applied in the form of heat, the steroid molecules of which the particle is composed tend to reach a more stable orientation. In the course of this rearrangement, the surface area of the particle is reduced.

The results of this work indicate that both the rapid freezing-sublimation drying method of preparing fine particles and the physical properties of the powders produced by this method, warrant investigation in greater detail.

Approved for Publication_____

Approved Dale E. Kuester

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