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Dissolution and crystal growth in aqueous suspensions of cortisone acetate

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The change in particle size distribution of cortisone acetate in aqueous (saline) suspensions has been examined using the Coulter Counter. Rate of growth under standardized conditions was calculated as increase of diameter per unit time. Solubility of a water-unstable crystal form has been shown to be greater than that of the stable form. Discussion of the results reveals that crystal growth is mainly initiated by polymorphic transformation. Lattice energies, heats of wetting and solution of the different crystal forms are thought to be the rate controlling factors.

CORTISONE acetate is known to exist in several polymorphic forms; their preparation, characterization, stability and interconversion have been described by Carless, Moustafa & Rapson (1966). Collard (1961), published photographs of 4 μ particles of cortisone acetate which changed to particles of up to 100 μ by suspension in water for less than 24 hr. These observations were related to the caking and sedimentation on suspension but no attempt has been made to study them quantitatively or to correlate them with definite changes in crystal form.

As it is well known that there is a greater tendency for the smaller particles to go into solution (Ostwald ripening), we decided to investigate the whole problem of dissolution and crystal growth in a simple system of micronized cortisone acetate in aqueous suspension rather than the more complex pharmaceutical formulations available. Such a study would have a direct relevance to the understanding of the reasons which lead to caking and difficult resuspendability experienced with stored preparations. It would also help the formulation of preparations of greater physical stability and, it is hoped, therapeutic efficacy.

Experimental

APPARATUS AND MATERIAL

A Coulter Counter "Model A", fitted with a 100 μ orifice tube and a 0·5 ml manometer (Coulter Electronics Limited, Dunstable) was used. The instrument was calibrated for particle size measurement using Silver Birch Pollen (diameter 24·4 μ). Throughout the analyses, the instrument was kept in a constant temperature room at 25° $\pm$ 1·5°.

A Unicam SP 800 recording ultraviolet spectrometer was used to compare solubilities and nucleation behaviour of the different crystal forms involved. 1 cm silica cells were used with distilled water as the solvent.

Micronized cortisone acetate Form II from Roussel Laboratories was used. This was identified as Form II by infrared spectroscopy (Carless & others, 1966).

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PROCEDURE

Preparation of electrolyte solution. 0·9% w/v solution of sodium chloride in distilled water was prepared. This was saturated by shaking with excess cortisone acetate, filtered through filter paper and then through a number 3 sintered glass Pyrex filter under atmospheric pressure.

Preparation of suspension. 100 mg of micronized cortisone acetate Form II was thoroughly mixed in a glass stoppered weighing bottle with 0·5 ml of 5% v/v solution of Nonidet P 42 (non-ionic wetting agent from Shell Chemical Company Limited) in 0·9% sodium chloride solution. This was then gradually diluted with 9·5 ml of the Nonidet sodium chloride solution. The suspension was immersed in an ultrasonic bath (Mini-Sonic by L & R Ultrasonics Ltd) for 2 min and then kept undisturbed in the constant temperature room, being shaken only briefly before taking a sample for analysis.

Counting technique. Because the process of dissolution and crystal growth is a dynamic one, it was necessary to complete the size analysis in a short time. Counting was therefore made at only five threshold values representing a wide range of sizes. This was favoured by the fact that the cumulative size frequency distribution by number could be represented by a straight line plot (see later). For each size analysis about 0·2 ml of the suspension was pipetted into 100 ml of electrolyte solution contained in the Coulter beaker and stirred at a speed sufficient to ensure uniform mixing. The sampling process took about 1 min; counting was then done. The time taken to complete a size analysis varied from 5–7 min. Size analysis was repeated at suitable time intervals until little change was observed in the size distribution. Background and coincidence corrections were made in the usual way.

Microscopical and infrared examinations were made at various stages during the process to observe gross changes in shape and size of the particles and to demonstrate the occurrence of a polymorphic change. The Nujol mull technique was used for infrared determinations, the crystals having been previously dried on filter paper at room temperature.

Ultraviolet spectroscopy. Excess micronized cortisone acetate Form II was suspended in water and shaken frequently. At various time intervals, portions of the suspension were filtered off through filter paper, then through a number 5 sintered glass Pyrex filter. The filtrate was collected, its ultraviolet spectrum determined and absorbance values recorded at the 244 mμ maximum.

The same experiment was repeated with larger crystals of Form II prepared by crystallization from benzene and with Form IV prepared by crystallization from 95% aqueous ethanol. Meanwhile, portions of the filtrate were kept and their spectra determined after longer time intervals to see if there was any change in absorbance while solutions were out of contact with solid particles. Portions of these were also centrifuged at 10,000 rev/min for 10 min, to see if there was any change in absorbance which could be due to fine particles that might have passed through the filter and which would be thrown out of suspension by centrifugation.

In another experiment, the filtrate from Form II was innoculated with excess Form IV crystals, shaken occasionally, filtered at various time intervals and the spectrum determined as before. The object of this experiment was to find whether the absorbance changed in the same way as it did in the presence of suspended Form II.

Growth in a high humidity atmosphere. This experiment involved suspending a sample tube containing a little micronized cortisone acetate Form II inside a larger tube containing some distilled water. The larger tube was cooled in carbon dioxide ice and acetone until the water was frozen, then the whole atmosphere above was evacuated down to $<0{\cdot}5$ mm Hg. The ice was then allowed to thaw at room temperature. Freezing and evacuation were repeated twice to remove all permanent and dissolved gases. The apparatus was then allowed to warm to room temperature and left for over a week maintaining what should be approximately 100% humidity. The idea was to try and increase the diffusion of water to the surface of the solid cortisone acetate. After this period, the solid was examined by infrared spectroscopy to see if it had changed form and microscopically to observe any change in shape or size. The same experiment was repeated using solid cortisone acetate coated with 5% by weight of a non-ionic surface-active agent "$C_{16}H_{33}[OCH_2CH_2]_9OH$" which was dissolved in ether and the ether evaporated while mixing with the solid. This was hoped to increase the wetting of the cortisone acetate.

Growth at higher temperatures. The growth process was investigated at 65° in a constant temperature water-bath. Samples which had previously undergone growth were also subjected to this treatment. The solid samples were separated after several hours of suspension and examined microscopically and by infrared spectroscopy.

Results

Particle size distribution data on number basis were found to be most suitable for the presentation of results because they were much more sensitive to size changes than those on weight basis. However, calculations on weight basis always confirmed that the greater part of the particle population was examined and provided for a check on mass balance. Plots of the percentage number cumulative frequency oversize on a log scale against the equivalent particle diameter showed a straight line relation over most of the particle size range, thus providing a convenient basis for comparison of results.

Nonidet P 42 (Shell Chemical Company) which has approximately 27% of a polyethylene oxide condensate as active material was used as wetting agent. Control experiments showed that the rates of crystal growth were practically unaffected by its presence.

The change in particle size distribution with time is shown in Fig. 1. After 6 hr no further change was observed.

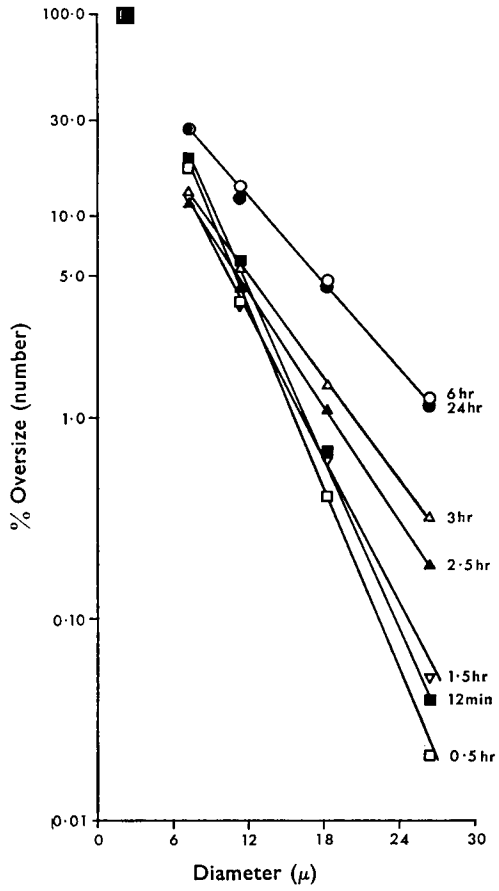

FIG. 1. Change in particle size distribution with time of suspension of cortisone acetate.

DERIVATION OF RATE OF GROWTH

To derive overall rate of growth, the principles adopted by Edmundson & Lees (1965) for solution rate studies were applied. Firstly, a plot of percentage number cumulative frequency oversize against time (Fig. 2) was established for diameters from 17 to 26 μ. This size range was selected because it represented the faster growing end of the distribution where readings from graphs of Fig. 1 type were feasible. Secondly, horizontal lines corresponding to various percentage cumulative counts were made to cut the curves in Fig. 2 in their steep portions. From the intersections, a plot of equivalent diameter against time (Fig. 3) was drawn, the slope of the straight lines in this plot being the rate of growth expressed as increase in diameter per unit time. Calculations on basis of Fig. 3 type of plot using the method of least squares showed that the rates of growth above 1, 2 and 3% cumulative count were 2·87, 2·28 and 1·86 μ/hr respectively.

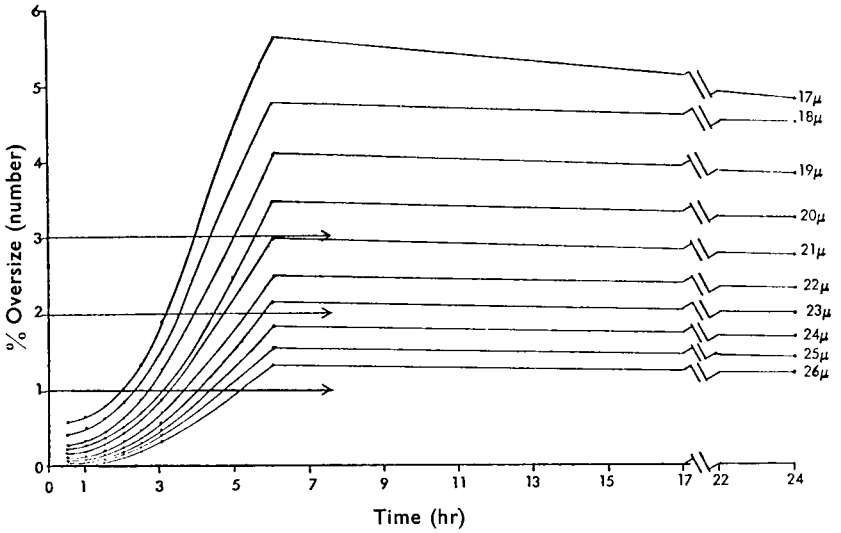

FIG. 2. Change in cumulative count with time of suspension of cortisone acetate. Curves from top downwards represent diameters of 17–26μ.

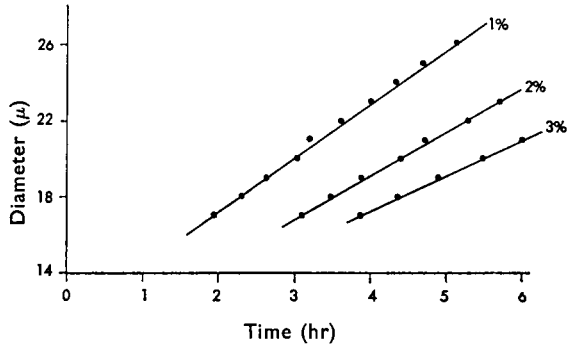

FIG. 3. Rate of growth (increase of diameter with time) in aqueous suspensions of cortisone acetate.

POLYMORPHIC CHANGE

The infrared spectra of cortisone acetate separated from the aqueous suspension showed a transition from the original Form II to a form originally reported as Form IV by Carless & others (1966). These workers noted that this water-stable form differed slightly from Form IV prepared by crystallization, as an additional adsorption band at 870 cm^{-1} was present. In the present paper this water-stable form is designated Form IV*. Unlike Form IV, which contained $\frac{1}{2}$ mole water/mole of cortisone, Form IV* contained a variable amount of water. Both these forms can be regarded as water-stable as the transition of IV → IV* in the presence of water is instantaneous and not accompanied by crystal growth.

Infrared spectroscopy also indicated that Form III was being formed from Form II suspended in water. This transition was difficult to detect

because once most of the solid had changed to Form III it rapidly hydrated to Form IV*.

The changes in size distribution of the cortisone acetate crystals reported in this paper were always accompanied by a change to Form IV*. Microscopical examination showed that the particles grew from small micron size round particles to long broad needles reaching 60 μ long.

ULTRAVIOLET SPECTROSCOPY

Micronized cortisone acetate (Form II) was shaken with water for periods of time from 20 min to $4\frac{3}{4}$ hr. The solutions were filtered and the absorbance of the filtrate was measured at 244 mμ. These results are shown in Fig. 4. When larger crystals of Form II were suspended, an increase in absorbance of the solution was first observed followed by a gradual decrease. The initial increase is presumably due to gradual saturation of the solvent which was not as fast as in the case of the micronized preparation. The filtrates saturated with Form II maintained the same absorbance for a long time even after centrifugation at a high speed. The addition of Form IV to the saturated filtrate resulted in decrease in absorbance at 244 mμ, with time. The difference in solubility between Form II and Form IV* (Form IV $\rightarrow$ Form IV* in water) is apparent from Fig. 4 where the absorbance falls from 0·857 for Form II to approximately 0·5 for Form IV*.

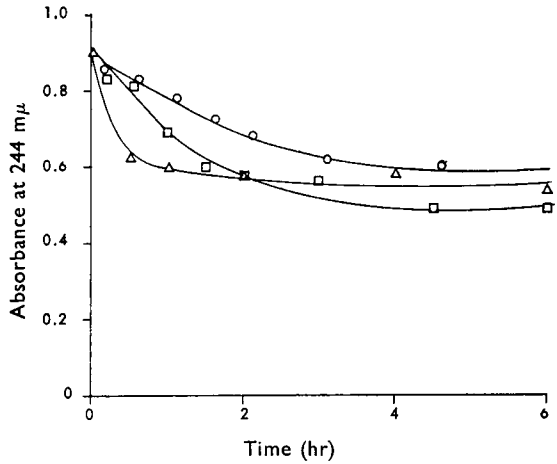

FIG. 4. Decrease in absorbance of a solution of Form II in presence of suspended Form II and Form IV. ○——○ in presence of suspended Form II (slight shaking). □——□ in presence of suspended Form II (mechanical shaking). △——△ in presence of suspended Form IV (mechanical shaking).

OTHER QUALITATIVE RESULTS

No growth or change of form was observed when crystals were suspended in a high humidity atmosphere or at high temperature. When crystals in suspension have previously undergone growth at room temperature, dehydration and reversion back to the original form was observed when the temperature was raised to 65°.

Discussion

To facilitate the discussion, it is convenient to list the processes thought to take place under the following headings: (1) Dissolution of small particles of the unstable Form II. (2) Polymorphic transformation of solid Form II to Form III. (3) Migration of molecules of cortisone acetate into their saturated solution. (4) Deposition of molecules of cortisone acetate onto nuclei of the water-stable Form IV*.

From ultraviolet spectroscopy evidence, dissolution is known to be very fast which is not unusual for particles of few microns size (see Fig. 4). The solubility of Form II is higher than that of Form IV as shown by their ultraviolet absorbance. Crystallization from a supersaturated solution with respect to Form IV is also fast on nuclei of this form (see Fig. 4). Both dissolution and crystallization are expected to be mainly diffusion controlled, according to the findings of Higuchi, Rowe & Hiestand (1963). One would expect, therefore, that the process which determines the rate of change would be the polymorphic transformation from Form II to III. The slope of the lines in Fig. 1 appears to stabilize at about 3 hr.

To investigate the significance of this initial 3 hr period in terms of dissolution and crystal growth, absolute frequency at certain diameter intervals was plotted against time. This was done by erecting vertical lines on a Fig. 1 type of plot to cut the size distribution lines at 2 μ

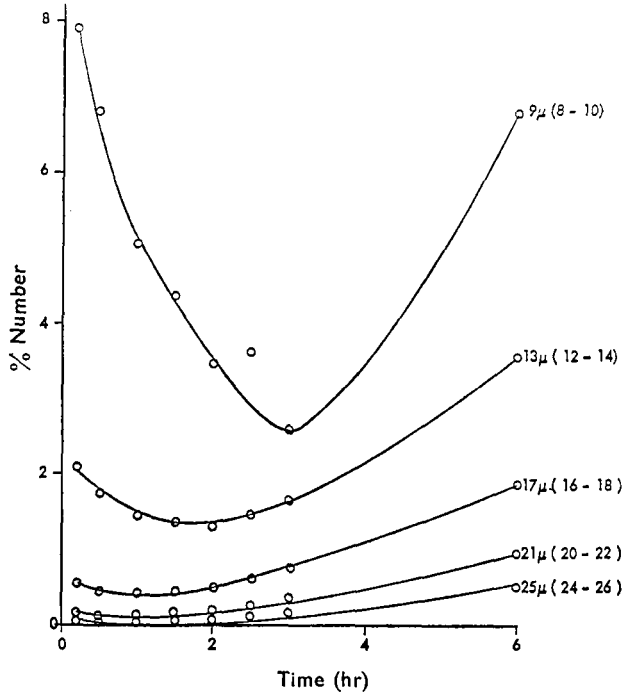

FIG. 5. Change in frequency percentage with time of suspension of cortisone acetate.

diameter intervals, the frequency at this interval being plotted against time (Fig. 5). This shows that for smaller particles there is a decrease in count down to a minimum which occurs at about 3 hr, followed by an increase in number presumably resulting from growth of still smaller particles. At large diameters the decrease is not significant and only an increase in count is observed at times over 3 hr. This can be explained as preferential solution of the small particles. The decrease in count with time represents mainly dissolution, although some nuclei of the stable form must have been formed during that time and consequently undergone growth. At the point of inflexion, there is a sufficient number of nuclei produced through the polymorphic process to make the rate of growth very nearly equal to the rate of dissolution. The increase in count with time represents predominant crystal growth affected by some solution process still going on. Complete interpretation was difficult to achieve because of the lower limit of particle size being counted by the Coulter Counter. Some very small particles were not counted and the use of a two-tube technique (using an additional smaller orifice tube) would have introduced serious errors because of the much longer time that would be necessary to complete the analysis. However, the uncounted particles represent only a small part of the population on weight basis.

The polymorphic transformation from Form II to III would very much depend on at least two significant energy values. One is the lattice energy and the other is manifested by the heat of wetting and heat of solution. The first of these is thought to be different for the two forms as suggested by their rates of change in the solid state and by differences in activation energies for transformations involving these forms calculated using infra-red spectroscopy (Moustafa, 1967). The second is expected to be different because of differences observed in the solubility of the two forms. Whether the difference between these two energy values for the two forms is positive or negative will determine the direction of the polymorphic transformation, and it is clear that this balance is in favour of the transformation from Form II to III in aqueous medium.

Higuchi & Lau (1962) in their theoretical treatment of growth rates advanced some equations to describe the rate of change of particle radius (or diameter) with time. One of these equations was,

$$\frac{da_1}{dt} = \frac{k_3 a_1}{4\pi\rho}$$

where t = time, ρ = density and a_1 = particle radius (equiv. vol. sphere).

Here the rate of change in radius is proportional to the radius. These authors thought of no simple physical system to exist for this case and they described a case which might approximate to it where the particles in the initial distribution are composed of aggregates of relatively uniformly sized primary particles and the growth rate is surface-area controlled.

Crystal growth in the cortisone acetate system appears to follow the above equation. The overall rate of growth in the system studied in this investigation was higher above 1% count level than it was above 3%.

Since particles of 17 μ average size will grow to an average size of 26 μ in the first case and only 21 μ in the second (see Fig. 3), then it can be stated that the rate of change in particle diameter is directly proportional to the diameter.

Water, being in contact with the surface of solid cortisone acetate particles provides, through heats of wetting and solution, for the shift of energy balance in favour of the transformation from Form II to III. This is contrary to the situation normally observed in the solid state where both of these important energy values do not show. The importance of the wetting and solution processes can further be appreciated by considering the fact that growth did not take place in an atmosphere of high humidity, even in the presence of a wetting agent. Growth did not take place in water at 65° and when a sample which had previously been grown at room temperature was introduced, it dehydrated and reverted to the original form (Form II).

Polderman, Bloo & Fokkens (1958) reported that the highest temperature to which a suspension of cortisone acetate could be heated without crystal growth is 60°. In contact with water, they stated, it is a considerable time before conversion to their water-stable form is complete and no needle-shaped crystals are obtained. The evidence from the present study so far contradicts these two statements. It is only when fairly large crystals (few mm diameter) of Form II are used instead of the micronized preparation that a delay in the transformation occurs. This could be attributed to the change in surface-area of the cortisone acetate. Polderman & others (1958) suggested the use of ethanol which presumably brings about some sort of crystallization producing Form IV which is more like the water-stable Form IV*.

Solubilities, rates of solution and nucleation properties of the different forms await further investigations. Results from the ultraviolet spectroscopy technique cited in the present study suffered from several difficulties and would indicate the necessity for special attention when a further study is being planned. Some of these difficulties arise because of variation of the amount of solid present and also its particle size, which in turn determine the number of nuclei available for crystallization. The rate of agitation also determines rate of growth.

It is clear from the above discussion that crystal growth in the cortisone acetate system under the conditions described is mainly initiated by the polymorphic transformation from Form II to Form III. Coarsening of suspensions by growth of the larger particles at the expense of the small ones is hardly responsible for the growth observed in a relatively short time.

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