groupes centrosymétriques, seront publiés prochainement.

Conclusion

La statistique dans le cas du cristal non centrosymétrique, abordée par Wilson (1949), Hauptmann & Karle (1953) et Vand & Pepinsky (1953), est présentée sous une forme nouvelle qui, espérons-le, nous approchera de la solution définitive du problème des phases.

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Crystalline Forms of Bovine Pancreatic Ribonuclease: Techniques of Preparation, Unit Cells, and Space Groups*

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Seven crystalline forms of bovine pancreatic ribonuclease have been prepared and characterized by X-ray diffraction. Two of these forms (I and VII) are complexes with nickel, and one (VI) is a complex with a dye, iodophenol blue. Methods of preparing each of the polymorphs are given. The observed lattice parameters are found to vary by small amounts on changing the composition (pH, etc.) of the medium from which the crystals are obtained. Some of the polymorphs were dyed by various members of the sulfonphthalein indicator series, while other polymorphs could not be dyed under similar conditions.

Techniques of crystallization

Methods of crystallizing bovine pancreatic ribonuclease from salt solutions at room temperature, or from aqueous alcohol at lower temperature, have been reported by Kunitz (1940). In the present work, techniques were developed for crystallizing ribonuclease at room temperature from aqueous solutions of various organic liquids. The crystals, which were required for an X-ray diffraction study of the crystal structure of ribonuclease, appeared in a number of distinct modifications, of which seven were characterized by X-ray diffraction data. The techniques reported here have made possible the reproducible production of these modifications, as their occurrence was found to depend on factors, such as the pH, the identity and concentration of the organic liquid used as a precipitant, and in some cases on the presence of small amounts of heavy metals or dyes.

The sources of ribonuclease for this work were: (1) A crude fraction from bovine pancreas precipitated from 0·7–0·8 saturated ammonium sulfate, purchased from Worthington Biochemical Company, and purified in this laboratory by either the method of McDonald (1948) or that of Kunitz (1940). (2) Lyophilized recrystallized salt-free ribonuclease purchased from Armour Laboratories. Different lots of the Armour ribonuclease differed in pH, but material giving a solution of a required pH could be obtained by dissolving the ribonuclease in water, adjusting the pH by addition of 0·1M NaOH or HCl, and lyophilizing again.

The methods of crystallization were:

(*A*) An aqueous solution of ribonuclease is frozen and then partially thawed. The organic liquid is added with gentle shaking, and the solution is then stored in a constant-temperature bath at 24–25° C. By use of this method of addition, both the effects of evolution of heat of mixing and of contact of nearly anhydrous organic liquid with the ribonuclease are avoided as much as possible.

(*B*) An aqueous solution of the organic liquid is chilled and poured on to solid lyophilized ribonuclease. The suspension is stored in the constant-temperature water bath at 24–25° C. The particles of ribonuclease

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normally swell to produce a viscous mass, from which crystals grow under certain conditions.

(*C*) The vapor of the organic liquid, which in this method must be volatile, is allowed to diffuse into the aqueous solution of ribonuclease at 24–25° C. This is conveniently done by suspending a small test tube containing the solution of ribonuclease in a larger stoppered test tube containing the organic liquid. In a few experiments, crystals were grown by evaporating water from a solution of ribonuclease containing a non-volatile organic liquid, but this technique has not given promising results thus far.

(*D*) The organic liquid is chilled and poured on to a frozen aqueous solution of ribonuclease. The frozen solution is allowed to melt without stirring, so that two layers are formed, and the layers are allowed to mix by diffusion at 24–25° C.

(*E*) A previously prepared crystal of ribonuclease is dried and crushed. The powder is mixed with a large volume of lyophilized ribonuclease powder. Then an aqueous solution of the organic liquid is added to a sample of the blended powder as in Method (*B*). Each fragment of crystal acts as a seed from which a new crystal grows at the expense of the amorphous material. Thus the number of crystals produced depends on the number of seeds added, and the ultimate size of the crystals depends on the amount of amorphous material added per seed. The production of crystals for X-ray diffraction studies, of dimensions about 0·5 mm., generally requires an extreme dilution of the crushed crystal, of the order of $1:10^6$, and the maintenance of conditions under which the rate of spontaneous formation of crystal nuclei is very low.

The methods found most convenient and reliable for preparing the various crystalline modifications are given in Table 2.

The utilization of any of these methods was possible only in the pH range from 4 to 8. Outside this range, the ribonuclease was transformed with more or less rapidity, in the solutions containing organic liquids, into a flocculent amorphous precipitate which was not observed to crystallize. This phenomenon is probably indicative of the type of change loosely referred to as denaturation.

Study of enzymatic activity

Selected crystals of modifications I, II, and V were washed free of mother liquor with 75 volume % alcohol, and subjected to enzymatic assay according to the method of Anfinsen, Redfield, Choate, Page & Carroll (1954). Each crystal showed ribonuclease activity of an amount consistent with the estimated volume of the crystal. The fact that modification I, a complex of ribonuclease with nickel, showed activity comparable to II and V is of interest. However, the activity was determined at pH 5, at which pH value ribonuclease is presumably not complexed by nickel, as modification I cannot be grown at such a pH.

Preparation of dyed crystals

When sulfonphthalein indicators were added to the crystallization mixtures, dyed crystals were obtained under various conditions. Generally, Method (*B*) or (*E*) was used, and a solution of aqueous alcohol containing the dye was added. In most cases the molar ratio dye: ribonuclease was unity. Larger amounts of dye usually, but not always, prevented crystallization.

Dyed crystals could also be prepared by immersing crystals in a dilute solution of a dye in 75 volume % alcohol, or a similar medium in which the crystals are insoluble. After a period of days or weeks, the crystals were dyed with a color about as intense as that of crystals grown from a dye solution. This is the more general method of dyeing, as it may be applied to all crystalline modifications of ribonuclease; it will be described further elsewhere, as will the application of heavy-atom dyes to crystal-structure determination by the isomorphous-replacement method.

Description of the crystal modifications

Ribonuclease I

This modification is a complex of ribonuclease with nickel. It is orthorhombic, space group $P2_12_12_1$, with 4 molecules per unit cell. Crystallographic data were reported by Fankuchen (1941) on a dry crystal of this modification grown by Dr M. Kunitz from ribonuclease material which had been handled in monel metal apparatus.

This modification was observed in our experiments only in samples to which $NiCl_2$ had been added to give a molar ratio of Ni : ribonuclease of from 1 to 9. One atom of nickel per ribonuclease molecule was apparently sufficient for the crystallization of this form; further addition of nickel had little effect on the crystallization. The pH range for appearance of this crystalline form was about 5·6–7·8, depending somewhat on the solvent. More acid solutions of ribonuclease plus nickel gave modifications III and IV, which may also be prepared in the absence of nickel, while more alkaline solutions gave modification VII (all described below).

The samples were usually made up with 22–25 mg./ml. ribonuclease, 50 volume % alcohol (*n*-propyl, iso-propyl, or tert.-butyl), or preferably 55 volume % 2-methyl-2,4-pentanediol. Methods (*A*) and (*D*) could be applied equally well. Method (*B*) could also be applied by using ribonuclease which had been lyophilized from a solution with pH and Ni concentration adjusted to values within the range indicated. Form I could not be grown by any method, even Method (*E*), in the absence of nickel. Since the seeds of modification I would not grow in nickel-free solutions, the requirement for nickel was verified.

The crystals of this modification, in contrast to those observed by Fankuchen (1941), have, in our experience, been thin plates bounded by {010} (as the

Table 1. *Crystallographic data for the modifications of ribonuclease*

(Crystals are in equilibrium with mother liquor unless indicated otherwise)

	Medium from which crystals were obtained	a (Å)	b (Å)	c (Å)	β (°)	Volume of unit cell (Å^3)
	Modification I; space group $P2_12_12_1$; $Z = 4$					
1	0·0078M $NiCl_2$, 50 vol. % iso-PrOH, pH = 7 (before adding $NiCl_2$) 6Ni^{++} per RNase	44·20	76·03	37·50	—	126000
2	55 vol. % MPD*, pH = 5·80, 3Ni^{++} per RNase	44·63	75·71	37·66	—	127300
3	55 vol. % MPD, pH = 6·60, 3Ni^{++} per RNase	44·48	75·74	37·71	—	127000
4	55 vol. % MPD, pH = 5·63, 9Ni^{++} per RNase	44·59	75·84	37·70	—	127500
5	55 vol. % MPD, pH = 6·02, 9Ni^{++} per RNase	44·48	75·74	37·64	—	126800
6	55 vol. % MPD, pH = 5·80, 3Ni^{++} per RNase, soaked in 75 vol. % MPD	44·28	75·84	37·52	—	126000
7	55 vol. % MPD, pH = 6·24, 3Ni^{++} per RNase, soaked in 75 vol. % MPD	44·32	75·79	37·61	—	126300
8	55 vol. % MPD, pH = 5·63, 9Ni^{++} per RNase, soaked in 75 vol. % MPD	44·44	75·62	37·52	—	126100
9	55 vol. % MPD, pH = 6·02, 9Ni^{++} per RNase, soaked in 75 vol. % MPD	44·28	75·65	37·60	—	126000
	Modification II; space group $P2_1$; $Z = 2$					
10	60 vol. % MeOH	30·37	38·39	53·34	106·39	59660
11	55 vol. % EtOH	30·49	38·32	53·30	105·94	59880
12	47 vol. % *n*-PrOH	30·39	38·46	53·45	106·18	59990
13	47 vol. % *n*-PrOH, soaked in 56 vol. % *n*-PrOH	30·43	38·41	53·32	106·12	59870
14	47 vol. % *n*-PrOH, soaked in 67 vol. % *n*-PrOH	30·38	38·39	53·26	106·01	59710
15	50 vol. % iso-PrOH	30·37	38·42	53·21	105·82	59710
16	50 vol. % tert.-BuOH	30·28	38·39	53·16	105·83	59450
17	50 vol. % tert.-BuOH, soaked in 90 vol. % tert.-BuOH	30·01	38·42	53·15	105·62	59020
18	10 vol. % 2-chloroethanol, 40 vol. % tert.-BuOH	30·37	38·44	53·27	105·83	59830
19	0·00187M bromcresol green in 50 vol. % tert.-BuOH (1 : 1 molar ratio)	30·33	38·44	53·23	105·90	59690
20	0·00187M bromphenol blue in 50 vol. % tert.-BuOH (1 : 1 molar ratio)	30·49	38·37	53·22	106·08	59830
21	55 vol. % 2,5-hexanediol	30·24	38·30	52·91	105·85	58950
22	55 vol. % 2-methyl-2,4-pentanediol	30·31	38·31	53·25	105·78	59500
23	65 vol. % monoacetin	30·24	38·37	52·99	105·87	59140
	Modification III; space group $P2_1$; $Z = 4$					
24	50 vol. % *n*-PrOH, pH = 5	42·91	45·38	77·2	114·31	137000
	Modification IV; space group $P6_222$; $Z = 24$					
25	47 vol. % *n*-PrOH, soaked in 67 vol. % *n*-PrOH	88·3	—	112·6	—	761000
	Modification V; space group $C222_1$; $Z = 8$					
26	60 vol. % γ-butyrolactone	29·17	61·34	126·0	—	225000
27	70 vol. % 1,3-propanediol	31·60	61·98	121·8	—	239000
28	38 vol. % 1,3-propanediol, 38 vol. % *n*-PrOH	31·0	61·6	121·4	—	232000
	Modification VI; space group $C2$; $Z = 4$					
29	0·00187M iodophenol blue† in 50 vol. % tert.-BuOH, pH = 5·2 (1 : 1 molar ratio)	70·60	38·99	51·65	103·96	137980
	Modification VII; space group $P2_1$; $Z = 2$					
30	55 vol. % MPD, pH = 7·05, 1Ni^{++} per RNase	46	28	46	102	—

* MPD = 2-methyl-2,4-pentanediol.

† Iodophenol blue = Na salt of tetraiodophenolsulfonphthalein

broad faces), {001}, {304}, and sometimes {100}. The crystals are often multiply twinned. The lattice parameters of this form, as grown under various conditions of pH, Ni concentration, and solvent, are given in Table 1. It is interesting to note that the parameters are not constant, but vary markedly with the pH and with the choice of organic liquid used. The nickel concentration, however, had little effect.

Some of the crystals grown from 55 volume % 2-methyl-2,4-pentanediol were transferred to a 75 volume % solution of this liquid and allowed to equilibrate. These crystals also showed marked changes in lattice parameters. The changes, while small, were reproducible, and outside the limits of error of measurement, which were estimated to be ±0·1% in each parameter. Crystals prepared under identical conditions always showed the same parameters, within these limits. In general, a decreased and c increased

Table 2. *Recommended methods for obtaining crystal modifications*

Polymorph	Method	Initial state of protein	Composition of sample
I	*A* or *D*	5% aqueous solution, $\geqq$ 0·00374M $NiCl_2$ (1 or more Ni^{++} per RNase), adjusted to pH 6·4	Add 244 μl. MPD* per 200 μl. sample of protein solution
II	*E*	Lyophilized RNase giving pH 5·0 in solution, with a trace of crushed II crystal	Add 1 ml. 50 vol.% tert.-butyl alcohol per 25 mg. sample
III	*D*	5% aqueous solution, pH = 5·0	Add 500 μl. *n*-propyl alcohol per 500 μl. sample of protein solution
IV	*A* (with long standing)	5% aqueous solution, pH = 5·0	Add 450 μl. *n*-propyl alcohol per 500 μl. sample of protein solution
V	*B*	Lyophilized RNase, giving pH 7·1 in solution	Add 1 ml. 70 vol.% 1,3-propanediol per 25 mg. sample
VI	*E*	Lyophilized RNase giving pH 5·0 in solution, with a trace of crushed VI crystal	Add 1 ml. of 0·00187M iodophenol blue† in 50 vol.% tert.-butyl alcohol per 25 mg. sample
VII	*D*	5% aqueous solution, 0·00374M in $NiCl_2$, pH 7·05	Add 244 μl. MPD per 200 μl. sample of protein solution

* MPD = 2-methyl-2,4-pentanediol.
† Iodophenol blue = Na salt of tetraiodophenolsulfonphthalein.

General remarks.—All preparations were at 25° C. The pH was adjusted with dropwise addition of 0·1N NaOH or 0·1N HCl with stirring, whenever necessary. Armour lyophilized salt-free ribonuclease was used; it was adjusted in pH and re-lyophilized whenever necessary. The dilution of crushed crystal in Method (*E*) was standardized by a few trial experiments to give a desired size of crystal. No salts were added except those indicated. All organic liquids were the purest grade commercially available, except 1,3-propanediol. This substance was treated with ion-exchange resin Amberlite IR-112 in the H^+ form for several hours at 100° C., shaken with soda lime, and distilled *in vacuo* in order to remove impurities present in the commercial material.

with increasing pH, while both of these parameters decreased on soaking the crystals in the stronger glycol solution. The changes in *b* were not significant. Similar variations were observed with other modifications, as described below. The minimum spacings for which X-ray diffraction spectra were observed were about 1·5 Å.

Ribonuclease II

This modification is monoclinic, space group $P2_1$, with two molecules in the unit cell; it has been described by Fankuchen (1945; see also Cohn & Edsall, 1943) and Carlisle & Scouloudi (1951). This modification appears to be the stable crystalline form of ribonuclease over a wide range of conditions, as it could be grown from a wide variety of solutions and in some experiments appeared as the product of transformation of another modification left in contact with its mother liquor. The lattice parameters observed for crystals of this form, as grown from several media, are given in Table 1. The estimated limits of error of *a*, *b*, and *c* parameters are $\pm$0·1%, and those of β are $\pm$0·02°. It is interesting to note that the lattice parameters, as with modification I, vary according to the medium from which the crystals were obtained. The changes in the lattice parameters were small, but reproducible, the greatest variation in volume being a little more than 1%. The minimum spacings for which X-ray diffraction spectra were observed were generally of the order of 1·5 Å, and in one case as short as 1·1 Å.

The reproducible variations in lattice parameters are interesting, but the explanation is not immediately obvious. They are not correlated with each other, nor with the molecular weights of the organic liquids used, as is indicated by the relatively large difference in parameters for crystals grown from *n*-propyl and isopropyl alcohol solutions. The variations may indicate slight changes in the orientations of the protein molecules.

Some of the ribonuclease II crystals were transferred from their mother liquors to stronger aqueous alcohol solutions, in which the crystals are essentially insoluble. It was found that the crystals remained transparent in 90 volume % *n*-propyl alcohol or tert.-butyl alcohol, but in more concentrated alcohols (about 95 volume % or greater) the crystals became opaque and crumbled. Lattice parameters of some of the crystals soaked in strong aqueous alcohols are given in Table 1. As may be noted from the unit-cell volumes, the effect of soaking in strong alcohols is a very small shrinkage.

Ribonuclease II could be grown over a wide pH range, from 4 to 7, but pH values from 6 to 7 were preferable in crystallizations with most of the precipitating alcohols. In this pH range modifications III and IV do not easily appear, and hence the crops of crystals were usually not contaminated with these modifications. The most suitable methods of preparing form II were (*B*) and (*E*) (after crushed crystals became available). Excellent large crystals (about 1 mm.) of form II were obtained occasionally by Method (*C*), but this method was not so reliable as the others. The proportions normally used were 25 mg. ribonuclease

to 1 ml. of liquid. By use of Method (*E*), crystals of this modification were grown not only from aqueous alcohols, but also from solutions of some of the glycols and other water-miscible organic liquids: 2,5-hexanediol, 2-methyl-2,4-pentanediol, 1,5-pentanediol, and monoacetin.

Crystals of modification II were also obtained by the same method (*E*) from mixtures of simple and halogenated alcohols in aqueous solution (e.g., from a solution of tert.-butyl alcohol and 2-chloroethanol in water), and from solutions of sulfonphthalein indicators in 50 volume % tert.-butyl alcohol. The latter crystals were grown under conditions with a molar ratio of indicator: ribonuclease = 1.

The crystals obtained from bromphenol blue, bromcresol green, and chlorphenol red were strongly dyed with the alkaline colors of the respective indicators, while the crystals obtained from the other, less acid, sulfonphthalein indicators were generally weakly dyed with the acid color (yellow). The measured lattice constants (Table 1) of dyed crystals did not differ greatly from those of undyed crystals. The crystal containing bromphenol blue appeared to be dyed more intensely than that containing bromcresol green. Since a crystal dyed with bromphenol blue did not show appreciable dichroism, it is improbable that the molecules of the dye are in parallel positions in the crystal.

The habits of crystals of form II were similar to those described by Carlisle & Scouloudi (1951), but the relative sizes of the faces varied somewhat with the medium from which the crystals were obtained. Often the crystals were elongated along *b* and flattened along *a*. In all cases, however, the developed forms were {100}, {011}, and {001} (the last very rarely absent).

Ribonuclease III

This modification is monoclinic, space group $P2_1$, with four molecules in the unit cell. This modification was often the first type of crystal observed in a crystallization. The crystals of form III grew rapidly as thin striated laths, elongated along *b*. The lattice parameters were not as reproducible as those of modification II, even for crystals prepared by the same method. Values from a typical crystal are given in Table 1. It should be noted that the volume per molecule of protein is greater than with the other modifications. The X-ray diffraction effects showed that the III crystals were normally twinned by rotation about the *a* axis; the striations are then probably due to multiple twinning. The minimum spacings for which X-ray diffraction spectra were observed were 2·8 Å.

Under some conditions of growth, form III is quite unstable. If the crystals are left in contact with the mother liquor, crystals of modification IV or occasionally of II begin to grow at the expense of the III crystals.

Modification III was best prepared by Method (*D*) in samples made up to 45–50 volume % *n*-propyl alcohol, 25 mg./ml. ribonuclease. (Method (*A*) gave somewhat smaller crystals.) The best pH range was from 4 to 5, while at pH values greater than 6 modification III was seldom observed. Since these conditions for optimum growth of form III are such that this form is highly unstable, it is necessary to transfer the crystals to more concentrated aqueous alcohol to prevent the subsequent growth of the other forms. Under conditions where form III is more stable, as in tert.-butyl alcohol, only minute crystals may be obtained, recognizable as III only by appearance. Isopropyl alcohol could also be used in preparing this form by Methods (*A*) or (*D*), but the crystals obtained were smaller than those from *n*-propyl alcohol.

Minute laths appearing to be form III could be obtained from any of the simple water-miscible alcohols by Method (*E*). Dyed crystals of modification III could not be obtained in experiments conducted by Methods (*A*) or (*B*) in the presence of sulfonphthalein indicators; either the crystals obtained were colorless, or no crystals were obtained (the usual case). A possible explanation of this phenomenon is that the dye molecules are adsorbed by ribonuclease molecules at the points at which they touch each other in the III crystals. If the dye molecules are strongly adsorbed by the ribonuclease molecules, they interfere with the contacts of the latter, so as to prevent the growth of crystals of form III.

Ribonuclease IV

This modification is hexagonal, space group $P6_222$, probably with 24 molecules in the unit cell. Photographically determined lattice parameters are given in Table 1 for a crystal which had been transferred from its mother liquor to 67 volume % *n*-propyl alcohol (for the sake of preservation of the crystal). The minimum spacing for which X-ray diffraction spectra were observed was 4 Å. This modification was often obtained from *n*-propyl alcohol solutions, and occasionally from isopropyl or ethyl alcohol, usually as a crop of hexagonal leaflets growing in a solution too dilute for crystals of III to be formed. This form was, however, observed sporadically at pH values up to 7. Growth of crystals of form II at the expense of IV was seen in a few cases, but the reverse transformation was never observed. Methods (*A*), (*C*) and (*D*) could be used in preparing this form with approximately equal success.

Ribonuclease V

This modification is orthorhombic, space group $C222_1$, with eight molecules in the unit cell. The crystals are normally rectangular parallelopipeds bounded by {100}, {010}, and {001}, although a bisphenoidal form is occasionally seen. This modification appears to be the stable crystalline form of ribonuclease in equilibrium with solutions of two particular substances

1,3-propanediol and γ-butyrolactone. It could be grown from either 65–70 volume % 1,3-propanediol or 60 volume % γ-butyrolactone, or even from mixtures of 1,3-propanediol and *n*-propyl alcohol in water (which gave twinned crystals of poor size and shape, unstable with respect to form II, however).

From all of these solutions, modification V grew spontaneously, fairly rapidly from the γ-butyrolactone solution, and more slowly from 1,3-propanediol; hence the glycol was preferred for the preparation of large crystals of form V. The solutions of these precipitating agents were found to deteriorate on standing to the extent that crystals of V could no longer be prepared. For this reason, aqueous solutions made up fresh from vacuum-distilled materials were found to be most reliable. The most suitable methods were Methods (*B*) and (*E*), although Method (*A*) could also be applied. No other modifications were ever found to appear in crystallizations from solutions of either of these reagents, in distinction to the crystallizations from 1,3-propanediol-*n*-propyl alcohol mixtures. The lattice parameters of form V, given in Table 1, show variation by several Ångström units, depending on the medium from which the crystals were obtained, although they were reproducible within 0·1% for crystals grown by a given technique. This variation is of a much greater magnitude than that observed with forms I and II, and may be an indication that the contacts between protein molecules are made through molecules of the glycol or lactone. The minimum observed spacing was 1·8 Å.

Crystals which looked like modification V could be obtained from some of the aqueous alcohols by Method (*E*), in which a crushed crystal of V was used. However, these crystals were unstable, and crystals of form II were observed to grow at their expense. Crystals of form V dyed with chlorphenol red could be prepared from 65 volume % 1,3-propanediol containing 1 mole of indicator per mole of ribonuclease.

Ribonuclease VI

This modification is monoclinic, space group $C2$, with four molecules in the unit cell. It may be obtained together with forms II and III by Method (*B*) from a solution of iodophenol blue (tetraiodophenolsulfonphthalein Na salt) in 50 volume % tert.-butyl alcohol. Ribonuclease and dye were present in 1 : 1 molar proportions in the sample. The crystals of VI are strongly dyed blue-violet, while those of form II are somewhat less strongly dyed, and those of form III are essentially colorless. The appearance of the crystals of II and VI is often quite similar, but form VI may be distinguished by its high pleochroism. Attempts to grow VI by Method (*E*) in the absence of dye, or in the presence of another sulfonphthalein (even bromphenol blue) were not successful. This modification is described further by Magdoff & Crick (1955), who have demonstrated a close structural relationship between forms II and VI.

Ribonuclease VII

This modification is monoclinic, space group $P2_1$, with two molecules in the unit cell. Like form I, this form is a complex of ribonuclease with nickel, but VII appears at higher pH values, from 6·7 to 8·6. At pH values about 7, both forms often appear simultaneously. As with modification I, one atom of nickel per protein molecule appears to be necessary, while addition of more nickel has little effect on the crystallization. Form VII crystallizes as needles, sometimes in spherulites, but more often as paired bundles of needles radiating in two opposite directions from a center. The latter mode of occurrence is both strange and quite characteristic of VII; in one sample the pair of bundles was found to be growing from the ends of a small crystal of I in directions nearly parallel to the *a* axis of I. The paired bundles of VII may, indeed, have all arisen from nuclei of I under pH conditions in which form I is unstable. At best, the crystals of VII thus far obtained are barely large enough for X-ray diffraction studies, and accurate values of the parameters could not be obtained. Observed lattice parameters are given in Table 1.

Detailed recommended procedures for preparing each of the polymorphs are given in Table 2.

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