

years, and many of these supervisors were rather antagonistic towards 'staff' specialists at the beginning of the course. The appreciation of the functions, aims and difficulties of the various specialists which the supervisors gained by meeting them on the courses seemed to lead to some modification of attitude.

When the supervisors as a body considered the 'problems' presented to them, discussion seldom rose above the level of a discursive exchange of views which tended to encourage the mutual confrontation of prejudices, often with elaborate rationalizations in support. On these courses the free discussions often seemed to reinforce the very attitudes they were designed to change. In so far as they led to a more detached approach, this seemed to be negative rather than constructive, and was marked by scepticism rather than by a willingness to face and to consider the merits of solutions which did not necessarily accord with the individual's preconceptions.

Another pattern of behaviour, superficially different but basically similar, emerged whenever supervisors took part in other types of group discussion. Members identified themselves closely with their syndicate, so that when the time came to report back, a debate rather than a discussion developed, and each syndicate tended to defend its own point of view regardless of the merit of contributions made by the other syndicate. Under the pressure of the report-back situation the syndicates tended to prepare a case to support a particular point of view, rather than to examine objectively the merits of a variety of points of view.

Further evidence of the shortcomings of the discussions on these courses was provided by the frequency with which the supervisors asked for a model answer to be given by the course leader or some other authority. When the individual members of the syndicates found themselves in agreement they were well satisfied, and felt that the problem before them had been solved. On the many occasions when there was disagreement lively discussion ensued during which each defended his own ideas and preconceptions and attacked those of others. All were quite confident that the correct solution had been achieved when agreement was reached, whereas, on the other hand, when disagreement was marked the group was often left in doubt as to the merits of the various solutions proposed. In the latter event, however, there would frequently be an appeal to arbitration, a request for some authoritative judgment against which these solutions could be evaluated.

An attempt was also made to determine to what extent the courses dealt with the actual problems which the supervisors experienced in their work.

The particular source of dissatisfaction which was mentioned most frequently was the relationship between the supervisor and his manager. Supervisors spoke in the main of their managers' remoteness or inaccessibility, of managers being out of touch with them and with shop-floor realities, and of a generally unsatisfactory state of communication between supervisor and departmental manager.

Those who considered that their relations with other supervisors were one of their main problems mentioned not only inter-departmental friction but also a lack of co-operation from colleagues in their own departments.

The courses appeared to achieve positive results in three directions. They were successful in improving the supervisors' knowledge of personnel policies and services, and thus should enable them to make a

more effective contribution to the smooth working of existing procedures. Concerning relations between supervisors and personnel officers, it cannot necessarily be assumed from the changed attitudes towards 'staff' personnel, which were evident towards the end of each supervisory course, that actual working relations would be improved, for the members of the personnel staff with whom supervisors normally have dealings are the subordinates of the managers who addressed each course. Finally, each course promoted improved relationships between the supervisors who took part in it.

On the other hand, it seemed unlikely that any other changes of an enduring nature in the behaviour or relationships of the supervisors would result. The evidence from the interviews suggests that many of the main problems of supervisors arise from their social relationships at work.

The investigators believe that while their studies stress the urgent need for more systematic evaluation of existing training arrangements, they also point to the desirability of a more experimental approach to training. The practitioner may find that a willingness to experiment in the development of training programmes may prove of greater value than a slavish adherence to the *ad hoc* course; it would also facilitate evaluation, for research should be more rewarding if it were possible to study systematically a variety of arrangements.

## ALPHA- AND BETA-FORMS OF POLY-L-ALANINE

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THE existence of folded and extended forms of synthetic polypeptides is now established beyond doubt<sup>1</sup>; but well-crystallized samples of the same polypeptide in both forms have not hitherto been obtained. We have recently found that poly-L-alanine can be produced in highly crystalline and well-oriented  $\alpha$ - and  $\beta$ -forms, and here we give a preliminary account of the chief features of their X-ray diffraction patterns. Poly-L-alanine is particularly important from the point of view of structure determinations, since the side-chains, consisting of single methyl groups, are restricted to two configurations corresponding to the extra-chain valencies of the  $\alpha$ -carbon atoms, apart from possible distortion.

The X-ray diagrams are shown in Fig. 1 *a, b*. The  $\alpha$ -diagram (Fig. 1*a*) is generally similar to that of poly- $\gamma$ -methyl-L-glutamate<sup>2</sup>. The equatorial reflexions fit accurately a hexagonal cell with  $d_{10\bar{1}0} = 7.40$  Å. As with poly- $\gamma$ -methyl-L-glutamate, it is not easy to fix the exact length of the *c*-(fibre) axis. The layer lines are close to the positions corresponding to  $c = 27$  Å., but there is a sharp meridional reflexion at 4.4 Å. which, if part of the same system, necessitates a longer repeat, for example, 70.4 Å. A similar reflexion at 4.3 Å. was found in poly- $\gamma$ -methyl-L-glutamate, in which the length of the *c*-axis is at least 43 Å. The 4.4-Å. reflexion in poly-L-alanine could only be observed when the specimen was tilted appropriately, because of the high orientation of the sample. It does not appear, therefore, in Fig. 1*a*. As we have already mentioned, such a reflexion cannot arise from an undisturbed  $\alpha$ -helix, and its

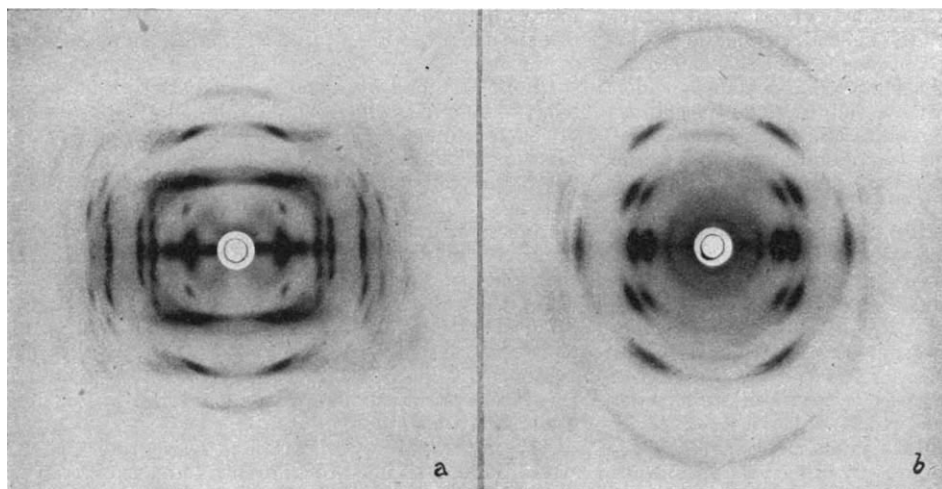


Fig. 1. X-ray diffraction patterns of (a) oriented  $\alpha$ -poly-L-alanine; fibre axis vertical; copper  $K\alpha$  radiation; cylindrical camera, nominal radius 3 cm.; (b) oriented  $\beta$ -poly-L-alanine. Other details as in (a)

presence in poly-L-alanine as well as in poly- $\gamma$ -methyl-L-glutamate shows that it is not produced by different configurations of flexible side-chains. It might, however, arise from distortion of some of the methyl groups due to difficulties in packing (see later). Only a slight distortion may be necessary, since the geometrical factor is very favourable for meridional reflexions.

No reflexion has been observed which necessitates increasing the base of the unit cell (in contrast with poly- $\gamma$ -methyl-L-glutamate), and there is therefore only one chain per unit cell.

The general distribution of intensity over the layer lines is strikingly similar to that in poly- $\gamma$ -methyl-L-glutamate<sup>2</sup> and agrees well with the calculations of Cochran, Crick and Vand<sup>3</sup> based on the  $\alpha$ -helix. The equatorial intensities also agree satisfactorily with those calculated from the co-ordinates of the atoms of the  $\alpha$ -helix derived from the most recently published dimensions of the polypeptide chain<sup>4</sup>. In addition to the 1.495-Å. meridional reflexion, a second spot appears on the same layer line on the first row line. The relative intensities of the two reflexions are approximately as predicted. The distribution of intensities on other layer lines is being compared with the calculated one, and as a result some changes in the atomic co-ordinates may be necessary.

The value  $c = 27$  Å. would, of course, correspond to an  $\alpha$ -helix having 18 residues in five turns. As mentioned earlier, however, it is necessary to consider larger values of  $c$ , and in our opinion  $c = 70.4$  Å. gives the best agreement between the calculated and observed positions of the layer lines. This would correspond to an  $\alpha$ -helix with 47 residues in thirteen turns. A consideration of the packing of  $\alpha$ -helical chains of poly-L-alanine shows that with  $d_{1010} = 7.40$  Å., steric interference between methyl groups on adjacent helices will arise. Further, the number of methyl groups per turn subject to this interference depends on the turn ratio of the helix, and for different helices goes down in the order  $18/5 > 29/8 > 47/13$ . The turn ratio of the helix may therefore be influenced by the packing of the side chains, and would be expected to be such as to give closest approach of neighbouring helices with minimum distortion of methyl groups. The slightly different turn ratio (29/8) observed for poly- $\gamma$ -methyl-

L-glutamate<sup>2</sup> is consistent with this idea.

An X-ray photograph of  $\beta$ -poly-L-alanine taken with the specimen tilted at an appropriate angle shows the strong meridional reflexion at 1.147 Å. which we have already reported<sup>5</sup>. This fixes the  $c$ - (fibre) axis as  $6 \times 1.147 = 6.88$  Å. The 1.147-Å. reflexion does not appear in Fig. 1b, since for this photograph the beam was normal to the specimen. The unit

cell appears to be orthorhombic with  $a = 4.79$  Å.,  $b = 10.7$  Å.,  $c = 6.88$  Å., and contains two chains. In a  $\beta$ -polypeptide the chains may be hydrogen-bonded into sheets in two ways: a 'parallel' sheet has all the chains parallel and running in the same direction, whereas an 'antiparallel' sheet has neighbouring chains running in opposite directions. Satisfactory models of either type can be constructed in which the  $c$ -axis is 6.88 Å. These sheets may be packed in parallel and antiparallel arrangements. The X-ray evidence shows that neither parallel sheets packed in a parallel manner nor antiparallel sheets can be present as major constituents of  $\beta$ -poly-L-alanine. The former would correspond to only one chain per unit cell, while the latter would require doubling of the  $a$ -axis. There is no evidence that a unit cell larger than that given above is necessary. It seems that the most probable arrangement is one in which sheets of parallel chains are packed in an antiparallel fashion.

Density measurements on these preparations are not satisfactory, because although the crystalline phases appear to be fairly pure  $\alpha$  or  $\beta$  as the case may be, infra-red spectra show that the amorphous regions contain appreciable amounts of the other component.

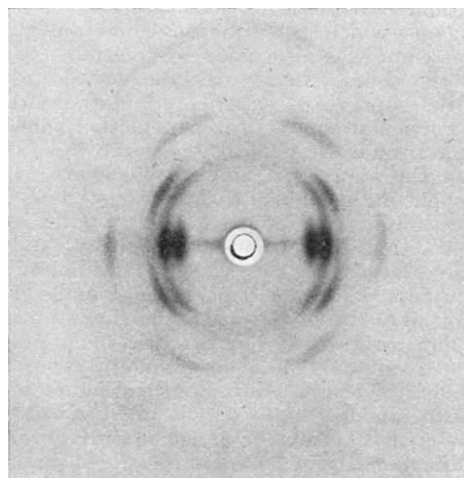


Fig. 2. X-ray diffraction pattern of Tussah silk. Other details as in Fig. 1 (a)

In conformity with results obtained on other polypeptides, poly-L-alanine shows different solubility behaviour in the  $\alpha$ - and  $\beta$ -forms. For example, the  $\alpha$ -polymer dissolves in dichloroacetic acid, but the presence of a small amount of  $\beta$  prevents solution.

In conclusion, we would point out the remarkable similarity between the X-ray diffraction photographs of  $\beta$ -poly-L-alanine and Tussah silk (Fig. 2). The spacings and the general distribution of intensity are almost identical in the two cases. There is, however, a complete absence in Tussah silk of the poorly oriented diffuse equatorial arc at 7.4 Å. arising from  $\alpha$ -material, some of which is always present in ' $\beta$ ' specimens of poly-L-alanine. The reflexions for Tussah silk may be indexed in terms of the two-chain orthorhombic unit cell suggested for  $\beta$ -poly-L-alanine, the difference in the cell dimensions in the two cases being less than 0.5 per cent. The only major difference in relative intensity is that of the equatorial reflexion near 5.35 Å., which is distinctly lower in Tussah. The amino-acid composition of Tussah silk is not known with any certainty, but the alanine content is apparently greater than in silk from *Bombyx mori*. It is reasonable to assume that the alanine in the former dominates the packing, although glycine and serine residues may also be present in the unit cell.

A detailed account of this work will be presented elsewhere in due course. [Nov. 9.]

<sup>1</sup> Bamford, C. H., Hanby, W. E., and Happey, F., *Proc. Roy. Soc. A*, **205**, 30 (1951); **208**, 407 (1951). Ambrose, E. J., and Elliott, A., *Proc. Roy. Soc. A*, **205**, 47 (1951).

<sup>2</sup> Bamford, C. H., Brown, L., Elliott, A., Hanby, W. E., and Trotter, I. F., *Proc. Roy. Soc. B*, **141**, 49 (1953).

<sup>3</sup> Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).

<sup>4</sup> Corey, R. B., and Pauling, L., *Proc. Roy. Soc. B*, **141**, 10 (1953).

<sup>5</sup> Bamford, C. H., Brown, L., Elliott, A., Hanby, W. E., and Trotter, I. F., *Nature*, **171**, 1149 (1953).

## CHLOROPHYLL CONTENT AND CARBON DIOXIDE UPTAKE OF STOMATAL CELLS

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THE guard cells of all light-sensitive stomata, including those of *Allium* (starch-free), appear to contain chlorophyll<sup>1,2</sup>, and the long-postulated importance of carbon dioxide tension in controlling stomatal movement is now well substantiated<sup>3-5</sup>. Yet, it has never been ascertained whether or not stomatal fixation of carbon dioxide, photosynthetic or otherwise, actually occurs. All the evidence cited in favour of the former<sup>3-7</sup> rests upon starch increases which are open to interpretation in terms of translocation from surrounding cells, and a recent attempt to demonstrate the stomatal production of oxygen by utilizing the technique of bacterial luminescence yielded only negative results<sup>2</sup>.

The epidermal chlorophyll content of *Tulipa gesneriana*, L. and the uptake of radioactive carbon dioxide by isolated epidermis of tulip, *Vicia faba*, L. and *Allium cepa*, L., have now been determined in an attempt to gain new information regarding the mechanism of stomatal movement. For chlorophyll determination, a sample of approximately three grams fresh weight of tulip epidermis was prepared from freshly harvested, healthy leaves of field-grown plants by a group of workers. The

	Epidermis			Ratio $a : b$	
	$a$	$b$	$a + b$	Epidermis	Leaf
% fresh weight ( $\times 10^{-4}$ )	2.46	1.36	3.82	1.81	3.45
Gm./guard cell ( $\times 10^{-12}$ )	1.81	1.00	2.81		

individual strips (0.5–1 cm.  $\times$  2–4 cm.) were carefully scraped and washed to remove adhering mesophyll and the chlorophyll extracted by repeated grinding with acetone and ether in a dark room. The final (anhydrous ether) extract was examined in a Beckman spectrophotometer (D.U.) and the quantities of total and chlorophylls  $a$  and  $b$  present calculated<sup>8</sup>. The presence of chlorophylls  $a$  and  $b$  was confirmed by paper chromatography. A similar analysis was carried out simultaneously using whole leaf tissue. The results are given in the accompanying table together with estimates of guard-cell chlorophyll which were calculated from the fresh weight/cm.<sup>2</sup> and stomatal frequency/cm.<sup>2</sup> of epidermis.

The validity of these estimates clearly depends upon the success with which the epidermal strips were freed of mesophyll. The stomata alone among the epidermal cells of tulip contain visible green plastids giving microchemical tests for chlorophyll. The difference between the ratios of chlorophyll  $a : b$  for the epidermis (1.81) and whole leaf (3.45) suggests that a part, at least, of the chlorophyll in the extract was stomatal in origin. It may be calculated from these ratios that the minimal chlorophyll content/guard cell is  $0.47 \times 10^{-12}$  gm. of chlorophyll  $b$ . However, the guard cells presumably also contain some chlorophyll  $a$  and their total chlorophyll content probably lies between 0.47 and  $2.81 \times 10^{-12}$  gm./cell. According to Freeland<sup>2</sup> the pigment in the epidermis (stomatal cells) of *Hymenocallis littoralis*, Salisb. is mainly chlorophyll  $a$ .

Similarly prepared epidermal samples of tulip were used for the measurement of carbon dioxide fixation. The strips, floated cuticle uppermost on water, were exposed for three hours at 26–27°C. in sealed 18-litre glass jars to carbon-14 dioxide in light (300 foot candles) and darkness respectively. The total carbon dioxide concentration was approximately 0.03 per cent by volume, and equal quantities of <sup>14</sup>CO<sub>2</sub> were supplied to each jar. Following exposure, the samples were washed in dilute acid and then with water to remove adsorbed <sup>14</sup>CO<sub>2</sub>, dried, ground to pass a sixty-mesh sieve and the activities determined directly at infinite thickness using a gas flow counter. Carbon dioxide uptake in the light (0.1181  $\mu$ C./gm. dry weight) was approximately twice that in the dark (0.0672  $\mu$ C./gm. dry weight). This result was confirmed by micro-autoradiographs of individual strips mounted on slides. Autoradiographs were made using Kodak X-ray film and NTB nuclear track plates. These yielded the information, revealed by microscopy, that carbon-14 had accumulated in the stomatal to a markedly greater extent than in the surrounding cells during the light exposure. This is illustrated in Fig. 1. Fig. 1A shows a micro-autoradiograph of a portion of a 'light' strip, while Fig. 1B shows the relative positions of radioactive centres and stomata respectively. In all radiographs active centres always corresponded exactly with stomatal positions, though usually some stomata (5 out of 27 in Fig. 1) showed no activity. This discrepancy was sometimes due to imperfect contact between the emulsion and the tissue strip, but