because the period in which the alteration in visible form of the sall was most rapid was complete at the point at which cotyledons were taken for analysis (after 4 days, germination). In view of this correlation, it seems possible that pectic polysaccharides are involved in the metabolic control of cell enlargement; their role could either be confined to the middle lamella[1], or be more general throughout the whole wall. The detailed chemistry of the process is not yet clear, but the general factors that might apply are outlined in the first part of this article and the evidence suggests that the chemistry is more complicated than has often been imagined in previous discussions. This function of the pectic polysaccharides would not exclude the possibility that enlargement is dependent on other biochemical changes as well.

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QUANTITATIVE GAS CHROMATOGRAPHY OF AMINO-ACIDS AS TRIMETHYLSILYL DERIVATIVES

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BECAUSE of its speed, accuracy and sensitivity, gas chromatography offers substantial advantages over other methods at present in use for the determination of amino-acids. A critical review of the literature on this problem so far is given by Lamkin and Gehrke[1]. In this review, however, the pioneering work of Ruhlmann on the synthesis and separation of the trimethylsilyl derivatives[2–5] is given little attention because of the reported instability of these derivatives. Although Ruhlmann himself is credited with having stated that these derivatives were too unstable for satisfactory quantitative analysis[6], we feel that this statement is entirely too broad and is possibly in error. It is the purpose of this article to present results showing that quantitative analyses of amino-acids are possible with at least three representative protein amino-acids.

(*A*) *Synthesis of amino-acid derivatives.* Three methods of synthesizing the trimethylsilyl (TMS) derivative of D,L-leucine were briefly explored as follows:

(1) The leucine was mixed with a 50-mole per cent excess of hexamethyldisilazane (HMDS) and refluxed until a clear solution was obtained and the evolution of ammonia practically ceased. The excess HMDS was removed by distillation at atmospheric pressure, the pot residue cooled, and the TMS-leucine distilled at 10-mm mercury pressure.

(2) The leucine was mixed with 100-mole per cent excess HMDS and refluxed as in method 1. The resulting solution was cooled to room temperature, diluted with about an equal volume of dry petroleum ether, and 50 mole per cent of triethylamine added to serve as a hydrochloric-acid scavenger. Finally, 50-mole per cent of trimethylchlorosilane (TMCS) was added dropwise with vigorous agitation, and the mixture stirred continuously for 2 h. This reaction mixture was then filtered under a nitrogen atmosphere to remove triethylamine hydrochloride and the clear filtrate distilled as in method 1. This method is a slightly modified version of the procedure proposed by Birkofer and Ritter[7].

(3) The leucine was mixed with a 30-mole per cent excess of trimethylsilyldiethylamine (TMSDEA) and refluxed on a Nester–Faust semi-micro spinning band still until the overhead temperature dropped to 55·5° C, the boiling-point of pure diethylamine. The diethylamine was then removed at a 10 : 1 reflux ratio. The overhead temperature remained practically constant until the theoretical quantity of diethylamine had been removed and then rose rapidly to 126·5° C, the boiling-point of pure TMSDEA. The excess TMSDEA was removed at this temperature, after which the pot was cooled and the crude TMS-leucine distilled at 10-mm mercury pressure as before. This procedure constitutes a minor variation of the procedure described by Ruhlmann[5]. The TMSDEA used in this work was synthesized in about 50 per cent yield by the method described by Hurwitz, Park and Benneville[8].

All fractionations of the TMS-leucine derivative were carried out on the Nester–Faust still mentioned in method 3. The purified product yield in method 1 was only about 33 per cent of theoretical and was contaminated with a white solid. Our tests indicated that this solid was pure leucine, confirming the observations of Birkofer and Ritter. These workers believed that this product formed in the distillate because of the incomplete conversion of the amine group to the TMS derivative; two moles of the partially silylated amino-acid reacting to yield a fully silylated product plus free amino-acid.

The two-step synthesis procedure (method 2) was designed to eliminate the difficulty mentioned here and did lead to slightly better yields and appreciably lower contamination of the distillates. However, method 3 was clearly superior to method 2 in our hands and gave nearly quantitative yields of pure TMS-leucine. The former

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Table 1. Physical Properties and Yields of Trimethylsilyl Derivatives

No.	TMS-Derivative of	Yield % of theory	BP ° C/ press (mm)	Relative retention time*
1	D,L-Alanine	61	76/15	1·00
2	Glycine	82	88/15	1·06
3	D,L-Valine	78	104/10	1·72
4	D,L-Leucine	87	108/10	2·07
5	L-Proline	76	87/5·5	2·63
6	D,L-Serine	50	114/4	3·07
7	D,L-Aspartic acid	95	113/4	4·70
8	D,L-Methionine	89	134/5	4·72
9	D,L-Phenylalanine	91	145/5	5·72
10	L-Lysine	81	147/4	6·67

* On 1 meter column of 10 per cent DC 200 on Gas-Chrom *Z*, programming from 92° to 200° C at 4 degrees/min, flow rate 100 c.c./min helium.

method was therefore used in synthesizing the amino-acid derivatives listed in Table 1. It should be noted in this connexion that the yields of derivative shown in this table are the actual yield of purified product obtained. No attempt was made to account for losses due to still hold-up, intermediate cuts, etc. Ruhlmann has indicated yields of 82–97 per cent for all the amino-acids tested, and we feel that his figures are probably more nearly correct than ours.

(*B*) *Gas chromatographic analysis conditions and procedure.* As noted in Table 1, columns of '*DC* 200' fluid (12,500 centistoke viscosity) on 'Gas-Chrom *Z*' gave good separations of most of the TMS amino-acid derivatives synthesized in this work. A number of other partition liquids were briefly tested and symmetrical peaks were obtained on such standard materials as '*DC* 710', 'Apiezon *L*', '*SE* 52' and '*QF*-1'. However, none of these partition liquids was any better than '*DC* 200' fluid. Attempts to use other standard partition liquids containing active hydrogen atoms, such as polyglycols or polyesters, failed to give recognizable peaks even after repeated injections or after attempts to react these partition liquids with excess TMSDEA before preparing the columns. In view of the distinct possibility that the TMS derivatives might prove to be too unstable or too reactive for gas chromatography anyway, it was decided to carry out a preliminary quantitative evaluation on the '*DC* 200' column. It was further decided to carry out these analyses under isothermal conditions to eliminate errors which might occur due to non-reproducible programming of temperature. D,L-Leucine, D,L-serine and D,L-aspartic acid were the amino-acids chosen for this work as being representative of a neutral, hydroxy-, and acidic amino-acid respectively. An Aerograph model *A*-350-*B* dual column temperature-programmed gas chromatograph equipped with a dual 4-filament detector cell was used with a Sargent model *SR* recorder. Helium was used as the carrier gas and 2 metres of standard 0·25-in. diameter copper tubing was used to contain the packing of 10 per cent '*DC* 200' fluid (12,500 centistoke viscosity) on 'Gas-Chrom *Z*'. The carrier gas flow-rate was set at 100 ml./min, and the column temperature at 160° C, in order to obtain a clean separation of the three amino-acids in an over-all analysis time of 8 min. Initially the injector and detector cell blocks were both set at 230° C, but a few preliminary semi-quantitative tests soon showed that better peak shapes and slightly more reproducible responses were obtained with on-column injection. The injector block temperature was therefore reduced to 160° C in subsequent quantitative work. In view of the known sensitivity of the TMS-amino-acid derivatives to traces of moisture, peak responses were also checked semi-quantitatively before and after the installation of a Matronic *XF*-100 gas chromatography scrubber in the carrier gas line between the helium tank and the gas chromatographic column. This unit is designed to remove traces of moisture, oil and dirt often found in commercial tanks of helium. Comparison of the data with and without the scrubber showed that the sample responses increased by nearly 40 per cent with the scrubber in place. This definitely showed the desirability of scrubbing the helium for this work even though it was not possible to detect any gain in weight in conventional magnesium perchlorate drying tubes after the passage of 30–40 l. of this gas through them.

Standard samples of the TMS derivatives of leucine, serine, and aspartic acid were prepared by injecting each of the pure derivatives into a dry, tared, and nitrogen-flushed vial through a self-sealing silicone-rubber septum. The vial was re-weighed after each injection, and finally a calculated volume of TMSDEA was injected to bring the final solution to the desired concentration and the vial again weighed. In this way the weight per cent of the final solution could be calculated accurately.

Reproducibility of sample size injection was checked by injecting replicate 2, 4, and 8 μl. samples into a tared vial. The vials were re-weighed after each injection so that both the reproducibility of injection and the average sample weight per injection could be calculated. The assumption was made in subsequent work that these average weights were also injected into the gas chromatograph. Thus, the size of each amino-acid component could be calculated from a knowledge of the weight-per-cent composition of the sample mixture. Calibration curves were then prepared by injecting triplicate samples of 2–8 μl. of several standard mixtures. Under the gas chromatographic conditions specified, the elution times of the three amino-acids chosen for analysis were 2·2, 3·3, and 6·8 min for leucine, serine, and aspartic acid respectively.

Finally, several mixtures of leucine, serine and aspartic acid were weighed out and converted to their trimethylsilyl derivatives by the following procedure: a mixture of 80–300 μmoles of each of the three amino-acids was suspended in 4–5 ml. of TMSDEA in a tared distillation flask. The suspension was refluxed on a Nester–Faust micro spinning-band still for 5–10 min, and then the resulting clear solution was slowly distilled so as to collect 2–3 ml. of distillate over a period of 50 min. Initially the overhead distillate temperature was 55°–60° C, but it gradually climbed to about 120° C at the end of the distillation time. The pot contents were removed from the still and the flask re-weighed so that the amino-acid content could be calculated. An aliquot of this solution was injected into the gas chromatograph and analysed under the conditions previously described.

The statistical correlations observed between the sample size of pure TMS-derivative injected and the average peak areas or peak heights are summarized in Table 2. Attempts were made to improve the precision of the peak area measurements by doubling the recorder chart speed, but no significant improvement was observed. It was concluded that the major cause of the relatively poor precision (95 per cent confidence limits were about ±10 per cent of the actual amount present) was due to the non-reproducibility of sample injection. This conclusion was confirmed by the following facts: (1) in every case where an unusually high or low response was obtained for leucine, correspondingly high or low responses were also obtained for serine and aspartic acid; (2) when analyses were calculated on a relative, rather than on an absolute, basis, the precision of the analyses was markedly improved (95 per cent confidence limits were ±0·6 per cent of the actual amount present).

Table 3 summarizes the results obtained on analysing four synthetic mixtures of pure leucine, serine and aspartic acid. Recorder peak height responses were used in this work since there seemed to be no advantage of the more complicated peak area determinations. Minor variations of the procedure described in the experimental section were used in these runs, and it is felt that the reproducibility shown in this table could be improved by strict adherence

Table 2. Statistical Correlation of Sample Size and Recorder Response for Pure TMS Derivatives*

Amino-acid	Peak heights		Peak areas	
	Corr. coeff.	*S.D.*	Corr. coeff.	*S.D.*
Leucine	0·995	0·019	0·995	0·019
Serine	0·995	0·019	0·996	0·018
Aspartic acid	0·995	0·022	0·996	0·019

* Range of sample sizes was 0·10–0·70 μmoles for each amino-acid.

Table 3. Analysis of Synthetic Amino-acid Mixtures

Sample No.	Component	Absolute amount in μmoles Present	Found	% Dev.	Relative amount (%) Present	Found	% Dev.
1	Leucine	101	81·4	−19·4	33·6	34·4	+2·4
	Serine	100	78·6	−21·4	33·2	30·2	−9·0
	Aspartic acid	100	76·5	−23·5	33·2	32·4	−2·4
2	Leucine	100	67·2	−32·8	32·8	31·9	−2·7
	Serine	80	54·1	−32·4	26·2	26·5	+1·1
	Aspartic acid	125	84·4	−32·5	41·0	41·7	+1·7
3	Leucine	91·1	66·7	−26·8	28·8	29·1	+1·0
	Serine	93·9	66·0	−29·7	29·7	29·4	−1·0
	Aspartic acid	131	92·2	−29·6	41·5	41·5	0·0
4	Leucine	138	114	−17·4	23·2	24·3	+4·7
	Serine	162	131	−19·1	27·2	27·9	+2·6
	Aspartic acid	296	225	−24·0	49·6	47·7	−3·8

to a single procedure. Nevertheless, the data in Table 3 are useful to demonstrate two points: (1) the average yield of amino-acid derivative via the procedure used was roughly 75 per cent for each of the three amino-acids; (2) the relative percentage composition of the mixtures could be calculated with an average precision of ±3 per cent (95 per cent confidence limit). The latter fact appears to substantiate the conclusion that all three amino-acids were being converted equally to trimethylsilyl derivative.

In summary, we feel that this work strongly indicates that the trimethylsilyl derivatives may yet prove to be the simplest and best derivatives for the gas chromatographic analysis of amino-acids. Work is continuing to extend this method to additional protein amino-acids and to refine the analytical procedures outlined in this article.

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CORRELATED ATOMIC COLLISIONS IN IRRADIATED CRYSTAL LATTICES

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When crystalline matter is bombarded with energetic radiation—protons, neutrons, α-particles, electrons, fission particles, etc.—the atoms of the lattice are disturbed and some are displaced, either singly or collectively, to form defects. The detailed examination of these processes is the physics of radiation damage, in which interest has been stimulated by the onset of the nuclear age, and with the realization of the importance of crystal defects in many solid-state devices[1,2]. Only comparatively recently, however, has close attention been paid to the fact that the atoms of an irradiated crystal are arranged in an ordered way on a three-dimensional lattice, though as long ago as 1957 Silsbee predicted that this would be of profound importance[3].

The way in which the regularity of the crystal can modify the problem is perhaps best seen in a qualitative way by considering a primary radiation damage event; that is, a collision between an incoming particle and a lattice atom. If the collision is a close one, then sufficient energy is communicated to the struck atom to displace it and, indeed, a cascade or shower of subsequent displacements may ensue. If the energy of the 'primary knock-on', however, is not so great, then the surrounding lattice is able to impose rigid conditions on the possible modes of momentum transfer. Thus, when the struck atom cannot penetrate the surrounding lattice it can only transfer energy and momentum to one of its immediate neighbours, and so the possibility of collision correlation becomes apparent. More particularly, where close-packed rows of atoms exist in the crystal, one may expect an energy pulse, or a sequence of collisions, to be propagated along them. This is the phenomenon of focusing, first suggested by Silsbee[3] and discovered in practice more recently through a series of elegant experiments performed by M. W. Thompson *et al.* at Harwell[4-6]. The bundle of energy which passes down a row of atoms is called a 'focuson', and it is said to have propagated by means of a 'focused collision sequence'. If the close-packed row of atoms is bordered by nearby atoms, then these also may exert some influence on the behaviour of the focuson, and an 'assisted focusing' process, which is very similar to the action which a simple thin converging lens exerts on a beam of light, may occur. When the nearby atoms lie close to the 'optic' axis, then the atomic lens is strong, focusing is enhanced, and the effective focal length is small. Moreover, if the focused collision sequence is sufficiently energetic, then it can be accompanied by a series of sequential atomic replacements. This is called a dynamic crowdion. Afterwards, of course, owing to the dissipation of energy to the atoms comprising the atomic lenses and because of thermal vibrations of all the atoms, a dynamic crowdion is degraded into a focuson, an interstitial atom is deposited, and the focuson fades away[7].

One other very important process derives from the existence of close-packed chains of atoms in the crystal, or rather from the open layers, or channels between them. If a primary knock-on enters and moves down such a channel, its interaction with the lattice will be small. It will travel long distances in an almost force-free space and will be constrained to stay there by an effectively infinite potential well created by the atoms in the close-packed chains. By analogy with the focuson, where the particular nature of the process was also emphasized through a high degree of energy localization, a primary of this kind has been called a 'channelon'[7]. The channelon, like the dynamic crowdion, but unlike the focuson, always transfers energy and mass.

The one single factor which perhaps best serves to distinguish between the phenomena of focusing and channelling is the energy at which they occur. Thus, while focusing is essentially a low-energy process (of the order of a few hundred electron volts in the close-packed metals), channelling is generally more favoured at higher energies (of the order of several keV): without any essential upper energy limit.

Computer methods. Both focusing and channelling are amenable to some kind of analytical treatment[7], but a comprehensive and accurate analysis is ruled out by the difficulties encountered in allowing for many simultaneous atomic interactions—the very core of the correlation problem. Moreover, our knowledge about the interaction potential itself is not very satisfactory, so that in many circumstances a numerical approach can be of greater value at this time. For this reason, Vineyard *et al.* at the Brookhaven National Laboratory have initiated computer calculations of radiation damage in face-centred cubic metals[8-11], and the work has since been extended by Erginsoy and others to the body-centred cubic structure[12]. The computer is asked to consider a crystallite containing a reasonably large number of atoms which interact with realistic forces. Atoms on the surface of the crystallite are supplied with extra forces simulating the reaction of