experiments is that branching in a high-polymer molecule is unlikely to be easily detected even though an authentic linear polymer of the same number average molecular weight were available for comparison. In a similar way, the variation of viscosity with concentration of the branched-chain molecules as compared with linear molecules is in qualitative agreement with the theories already put forward, but the results are not nearly so clear cut as the theories would predict.

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Catalytic Poisons and Magnetic Susceptibility

The alkyl sulphides are powerful catalytic poisons for palladium, and the nature of the adsorption link is therefore of great interest. On chemical grounds, Maxted[1] has suggested a co-ordinate link from the sulphur atom to the metal. Here we record some preliminary measurements on the change in magnetic susceptibility of palladium due to adsorption of dimethyl sulphide. We conclude that electrons from the sulphur atom enter the *d* band of the metal (in an analogous way to the electrons from *dissolved* hydrogen atoms[2]).

A finely divided sample of palladium powder (prepared by reduction of palladium chloride in glycerine by hydrogen gas at 150° C.) was evacuated in an ampoule at 150° C., sealed off, and its susceptibility measured by the Sucksmith method[3]. The ampoule was then reattached to the vacuum apparatus and the palladium saturated with dimethyl sulphide gas at 20° C. The amount adsorbed was measured, the ampoule resealed and measured in the magnetic balance.

Column 2 of the accompanying table shows the change in susceptibility of the palladium powder expressed as a percentage change on the original value χ. Column 3 gives the percentage change in χ due to the effect of adsorption on the palladium alone; that is, it is the value in column 2 *minus* the calculated diamagnetic susceptibility of the adsorbed dimethyl sulphide.

$\frac{100\ a^*}{b}$	$100\ \frac{\triangle\chi}{\chi}$	$100\left(\frac{\triangle\chi}{\chi}\right)_{Pd}$	$100\left(\frac{\triangle\chi}{\chi}\right)_{calc.}$
27	10·1	8·0	13·5
23	7·9	6·0	11·5

* a moles dimethyl sulphide adsorbed on b gm. atoms palladium powder.

The effect of the dimethyl sulphide is outside the experimental error of 2 per cent on χ, and is of the right order to be expected if one electron from the dimethyl sulphide enters the *d*-band of the metal. Since dimethyl sulphide is an easily condensable gas, the adsorption will correspond to *at least* two monolayers; that is, a van der Waals layer on top of a chemisorbed layer. Only the chemisorbed layer will affect the susceptibility, and this will contain *up to* half the adsorbed quantity in column 1. At this stage we can do no more than assume that a chemisorbed molecule transfers an electron to a vacant surface *d* orbital, and thus the susceptibility of the surface palladium atoms is reduced to zero (there is 0·55 mole in the *d*-band per palladium atom[2]). This would then yield the calculated decrease in susceptibility shown in the last column.

It is not worth discussing the discrepancy between observed and calculated figures until experiments with clean surfaces have been made. The main point of contrast is that if the electrons entered the *s*-band of the metal, the susceptibility change would have been immeasurably small.

It is hoped to develop this experiment along more quantitative lines, since it offers an approach to an important aspect of adsorbed layers. These preliminary measurements were made in the Physics Department of the University of Bristol, and we are grateful to Drs. H. Heitler and L. C. Jackson for their help and advice.

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Synthetic Pentapeptides Related to Gramicidin-*S*

The polypeptide nature of several antibiotics is of considerable interest. As recent work in this field overlaps to some extent with our own, we wish to give a preliminary account of our studies on synthetic peptides related to gramicidin-*S*.

Synge[1] extended the limited data published by Russian workers[2–4], and suggested that gramicidin-*S* was the hydrochloride of a basic peptide with a stoichiometric minimum unit containing equimolecular proportions of the five amino-acids, *L*-ornithine, *L*-proline, *L*-valine, *L*-leucine and the unnatural *D*-phenylalanine. Each stoichiometric unit contained one free amino group but no free carboxyl groups. The free amino group was later identified[5] as the δ-amino group of ornithine, and gramicidin-*S* was formulated as a cyclic peptide of five amino-acids linked through their α-amino and carboxyl groups. Degradative studies involving the technique of partition chromatography furnished strong evidence for the structure [α-(valyl-ornithyl-leucyl-phenylalanyl-prolyl-)]$_n$ [6]. On the basis of the physical information so far available, gramicidin-*S* is best formulated as a cyclopentapeptide, or as a cyclodecapeptide where the above unit occurs twice in a closed peptide chain.

In the hope that some light might be thrown on the origin of the striking antibiotic action of this peptide, we decided to carry out parallel syntheses so as to obtain two pentapeptides with phenylalanine present in both its optically active forms. Numerous alternative routes to such a pentapeptide might be envisaged. After several different combinations had been tried, it was decided that for ease of handling, which was a dominant consideration, synthesis of a tripeptide from leucine, phenylalanine and proline, and the coupling of this tripeptide with a dipeptide from valine and ornithine, would have a reasonable chance of success. For all syntheses carbobenzoxy derivatives were employed according to Bergmann's

general procedure, and coupling was effected in each case through the acid azide.

Cbzo-L-phenylalanine hydrazide was converted to the azide, coupled with *L*-proline ethyl ester, and the carbobenzoxy group removed by catalytic reduction. The dipeptide ester with *Cbzo-L*-leucine azide gave *Cbzo-L*-leucyl-*L*-phenylalanyl-*L*-proline ethyl ester. The acylated tripeptide ester could not be crystallized, but analysed satisfactorily (found: C, 67·2; H, 7·3; N, 8·18; theory: C, 67·1; H, 7·3; N, 7·84 per cent). It was used for coupling with the appropriate dipeptide without further purification.

For the synthesis of the isomeric tripeptide a modified procedure was used which gave crystalline intermediates. *Cbzo-L*-leucine azide coupled with *D*-phenylalanine methyl ester to give *Cbzo-L*-leucyl-*D*-phenylalanine methyl ester, m.p. 110°. The hydrazide (m.p. 170°) from this ester gave an azide which was condensed with *L*-proline methyl ester; the resulting acyltripeptide ester was purified as the hydrochloride after removal of the carbobenzoxy group. The tripeptide methyl ester hydrochloride $[\alpha]_D^{20} = -38{\cdot}9°$ [anhydrous methanol, $C = 2{\cdot}0$] melted at 240°.

The two optically isomeric tripeptide hydrochlorides were tested *in vitro* against *Staphylococcus aureus*, *Streptococcus hœmolyticus* and *Escherichia coli*. Both peptides inhibited growth at a concentration of 1–2 mgm./ml., but were inactive at lower concentrations.

For preliminary experiments on open-chain pentapeptides we used the readily obtainable *DL*-ornithine rather than the rarer *L*-ornithine, as we did not consider that use of an optical mixture of *L*-valyl-*L*-ornithine and *L*-valyl-*D*-ornithine would affect the validity of our conclusions. *Cbzo-L*-valyl-δ-*Cbzo-DL*-ornithine methyl ester was prepared by the azide method and crystallized readily, m.p. 150°. This was converted through the hydrazide to the azide, and coupled with both the tripeptide esters already described. Both the dicarbobenzoxypentapeptide esters were difficult to handle; they set to stiff gels when attempts were made at recrystallization. After repeated precipitation of one gel, a product, m.p. 128–132°, was obtained which analysed satisfactorily for *Cbzo-L*-valyl-δ-*Cbzo-DL*-ornithyl-*L*-leucyl-*L*-phenylalanyl-*L* proline ethyl ester (found: C, 65·0; H, 7·4; N, 9·85; theory: C, 65·2; H, 7·2; N, 9·5 per cent). After five further 'crystallizations' from aqueous methanol, the small quantity of material remaining melted at 164° and again analysed satisfactorily.

The second, isomeric acylated pentapeptide ester melted at 198–200°, after purification by the same means, and analyses were satisfactory for *Cbzo-L*-valyl-δ-*Cbzo-DL*-ornithyl-*L*-leucyl-*D*-phenylalanyl-*L*-proline methyl ester (found: C, 64·5; H, 7·1; N, 10·0; theory: C, 64·8; H, 7·1; N, 9·7 per cent). As a final check on the identity of the two peptides, these were hydrolysed and the hydrolysates subjected to chromatographic analysis on paper by the usual technique[7]. The presence of the required five amino-acids was demonstrated by comparison with an artificial mixture.

The above work was essentially of an exploratory nature and was carried out on the smallest scale consistent with the requirements of the biological tests. We are now repeating the synthesis of the *LLLDL*-pentapeptide on a larger scale using *L*-ornithine so as to eliminate any doubts about the optical purity of the final product.

No significant difference in antibacterial action was apparent between 'natural' and 'unnatural' isomers at either the tripeptide or the pentapeptide stage. These results support the view already expressed[8] that antibiotics containing *D*-amino-acids are active, not because they have this character in common, but rather in virtue of their specific structures, of which the *D*-amino-acids must be regarded as an integral part.

Crowfoot (private communication) has expressed the opinion from a study of models that *D*-configuration of some of the constituent amino-acids in a peptide such as gramicidin-*S* would facilitate cyclization. It is thus reasonable to postulate that the activity of this peptide is a function of its cyclic nature. When larger supplies of open-chain pentapeptide become available, we hope to study the effect of polymerization and, if possible, of cyclization, on antibacterial action. A full account of this work will be published elsewhere.

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Reversion: a New Procedure in Absorptiometry

Many metals form coloured complexes with dithizone, and the successful use of this reagent for estimating one of them in a mixture demands a careful selection of organic solvent, a rigorous control of *p*H and other variables such as duration of shaking, nature and amount of neutral salts present, the concentration of excess dithizone, and the concentration of 'masking' reagents capable of competitive complex formation with interfering metals[1]. The coloured metal complexes (yellow, red, violet, etc.) modify the colour of the green solution containing excess dithizone giving a 'mixed-colour' extract, and by absorptiometric measurements the proportion of metal complex can be estimated with reference to calibration curves obtained by treating known amounts of metal by the standardized procedure. Such curves are commonly valid only for one initial concentration of dithizone, and error may be introduced in view of the well-known impermanence of this reagent. The theoretically obvious solution for such systems with two coloured components is to measure the transmittency at two appropriate wave-lengths; but this involves a burdensome amount of preliminary work for, in effect, a family of calibration curves must be constructed to cover all possible variations in reagent concentration and in the amount of metal taken for analysis[2].

In a systematic study of the estimation with dithizone of traces of mercury in biological