graphy (Figs. 1-4), 20 μ m. protein being detectable. Protein degradation products do not cause a 'protein error' with the above indicators and, therefore, are not recorded on the chromatogram. Since neither bromothymol blue nor tetrabromophenolphthalein ethyl ester contains nitrogen, we are exploring the possibility that quantitative analysis of protein mixtures may be achieved by nitrogen determinations of the spots.

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Homolytic Aromatic Substitution

THE experimental facts and their theoretical interpretation for aromatic substitution by heterolytic mechanisms, including both electrophilic and nucleophilic reagents, are now well established. Although the possibility of homolytic aromatic substitution was recognized sixteen years ago¹, the absence of reliable quantitative data has hitherto made any comprehensive theoretical interpretation both difficult and speculative. Examples of homolytic aromatic substitution have in recent years been considerably extended and now include as substituting agents aryl radicals (from certain diazo compounds or acyl peroxides²), methyl radicals (from acetyl peroxide³, lead tetra-acetate⁴, phenyliodosoacetate⁵ or the electrolysis of acetates⁶), halogen atoms (in non-catalytic high-temperature halogenation⁶) and hydroxyl radicals (from Fenton's reagent⁷, the action of ionizing radiations on aqueous solutions⁸, or the irradiation of aqueous solutions of ion pair complexes⁹).

In all substitution reactions involving free radicals on which quantitative data are available, the atom or group *R* in C_6H_5R is invariably *ortho-para* directing in type, as was, indeed, recognized by Grieve and Hey¹; but the formation of the *meta* isomeride is not entirely suppressed except perhaps in the case of phenol. The hydroxylation of nitrobenzene by means of hydroxyl radicals appears to give 20-30 per cent of *m*-nitrophenol^{7,8}. The action of aryl radicals ($C_6H_5^{\cdot}$, $pCH_3.C_6H_4^{\cdot}$, $pBr.C_6H_4^{\cdot}$), whether prepared from the diazo hydroxide, the nitrosoacetylamine, the 1-aryl-3:3-dimethyltriazene, or the acyl peroxide, on nitrobenzene gives about 10 per cent of the 3-aryldiphenyl in each case¹⁰. In the hydroxylation of benzoic acid the formation of the three isomeric hydroxybenzoic acids in the approximate molar ratio *o*:*m*:*p*:2:2:1 has been reported⁹. From these results it appears that the differences displayed in the reactivities at the *ortho*-, *meta*- and *para*-positions to a substituent atom or group *R* are less accentuated in homolytic than in heterolytic substitution reactions. We classify all those atoms and groups as *ortho-para* directing which give rise to not more than 40 per cent substitution at the *meta* position.

The related problem of the influence of substituent atoms or groups on the velocity of homolytic substitution has been investigated by means of competitive reactions, and the results show that all such atoms or groups activate the nucleus^{11,12}. In mono-substituted benzene derivatives the nitro-group shows the greatest activating influence, nitrobenzene being about three to four times as active as benzene. This observation is in agreement with the well-known effect of aromatic nitro-compounds in retarding and inhibiting polymerization¹³. Chlorobenzene has been found to react at a rate approximately 1.3 times that of benzene, while the rate of substitution in pyridine is very nearly equal to that in benzene. Here again the effect of substituent atoms or groups upon the reactivity of the nucleus as a whole towards attack by free radicals is less marked than in heterolytic substitution reactions.

Quantum mechanical interpretations of these results have been put forward by Wheland¹⁴ and by Coulson¹⁵, and close agreement with the experimental facts is obtained. So far as present knowledge goes, it is thus possible to recognize two criteria which serve to differentiate these reactions from the better-known reactions of aromatic substitution involving electrophilic or nucleophilic reagents. In homolytic aromatic substitution, all substituents both activate the nucleus and are *ortho-para* directing. The influences which control these phenomena, however, are free from Coulombic forces and are therefore less powerful than those which operate in heterolytic substitution reactions.

Further details of this work and the logical extensions thereof will be published elsewhere.

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Contaminant of Commercial 3:4-Benzpyrene

IN their studies of the metabolic products of 3:4-benzpyrene, Berenblum and Schoental¹ separated by chromatography a material ('fraction V') which had a fluorescence spectrum of much shorter wavelength than those of benzpyrene and its simple derivatives. The same spectrum was later given by a chromatographic fraction from the products of oxidation of 3:4-benzpyrene with osmium tetroxide². It was then noted that the spectrum resembled that of 1':2':3':4'-tetrahydro-3:4-benzpyrene³.

This led to an examination of the commercial sample of benzpyrene used in both these investiga-