chain is terminated and the completed chain released from the ribosome-*m*RNA complex to which it is bound by the *t*RNA molecule carrying the last amino-acid incorporated. There must be a mechanism for reading the chain terminating codon (three codons UAA, UAG, and UGA can code for chain termination) and cleaving the last peptidyl-*t*RNA bond. In *Biochem. Biophys. Res. Comm.*, 28, 773 (1967) he describes an experimental system devised to give a rapid assay for chain termination, and in *Proc. US Nat. Acad. Sci.*, 58, 1144 (1967) he reports the discovery of an enzyme required for termination.

The assay for termination employs the RNA from an amber mutant of the coat protein gene of the RNA bacteriophage *R17*. This mutant has an amber codon, UAG, at the position of the sixth amino-acid, a glutamine residue, in the *R17* coat protein molecule. In a cell free system the mutant RNA directs the synthesis of a small *N*-terminal peptide of the coat protein with the sequence *N*-formylmet-ala-ser-asp-phe-thr: the next amino-acid in the coat protein is glutamine, but with the mutant RNA the amber codon causes premature termination of synthesis and the release of the hexapeptide. The great virtue of this system is that the unterminated peptide still attached to the peptidyl-*t*RNA can be distinguished from the terminated peptide released from the *t*RNA and the amounts of each measured. Furthermore, starving the system for any one of the six amino-acids in the peptide arrests the translation machinery before it reaches the UAG codon so termination can be controlled.

Exploiting this procedure, Capecchi isolated a complex of ribosome, *m*RNA and pentapeptide attached by the phenylalanyl *t*RNA simply by starving for threonine. With this he has been able to define the conditions necessary for termination; incubating the complex under suitable conditions with or without threonine or supernatant enzymes showed that to get release it is necessary but not sufficient to complete the peptide by incorporating threonine and that release is not solely dependent on the transfer enzymes required to move the ribosome from the threonyl codon to the UAG terminating codon. Release does, however, depend upon the presence of a supernatant factor. This factor, with a molecular weight of between 40,000 and 50,000, sediments between 3·5*S* and 4·5*S*. Because its releasing activity is not affected by RNase, it cannot be an RNA-protein complex. Apparently the substrate of the release enzyme is the ribosome-*m*RNA-peptidyl *t*RNA complex so that it must act directly at the ribosome and not cleave the *t*RNA from peptidyl-*t*RNA already released into the supernatant, which was the other possibility.

Capecchi does not yet know whether the release enzyme works alone or in conjunction with other factors, in particular a hypothetical chain termination *t*RNA which reads the termination codon but does not carry an amino-acid. Many people, including Capecchi, have searched in vain for such a *t*RNA species, and although negative results can never exclude the possibility that such *t*RNA exists it is reasonable to think about alternative models in which the terminating codons are read either by the ribosome or the release enzyme or in which nonsense codons function simply because they cannot be read at all. Further characterization of the release enzyme could well decide the issue.

Chlorinated Hydrocarbons in British Wildlife

by

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Polychlorobiphenyl compounds have been detected in British wildlife. In birds' livers and eggs they are often in greater quantities than organochlorine pesticide residues. Polychlorobiphenyls are known to be toxic, and their detection in wildlife raises the question of the adverse effect they may have.

When wild birds' eggs or the livers of British wildlife are examined by gas-liquid chromatography (GLC) using electron-capture detection, peaks corresponding to such organochlorine pesticides as BHC, dieldrin and DDT, and their metabolites and breakdown products, often appear on the resultant chromatograms. These peaks sometimes represent amounts which are significant from a toxicological point of view, but the sensitivity of modern instruments also enables completely insignificant amounts to be detected. Analysts concerned with the determination of organochlorine pesticides in wildlife, however, have long been aware that it is not unusual for an additional series of as many as ten peaks, corresponding to unidentified compounds, also to appear on these chromatograms[1-4]. Little is known of the structure and origin of these compounds, but Holmes[1] established that they are organochlorine in nature, with a substantial chlorine content, and consequently possess electron-capturing properties.

On silicone or 'Apiezon' GLC columns, the series of peaks for these compounds normally begins at the point at which *pp'*-TDE and *pp'*-DDT are emerging from the columns and extends, in terms of retention times, to four or five times that of *pp'*-DDT, which is the last of the commonly occurring pesticides to emerge.

Before examination by GLC, samples have normally been subjected to a fairly rigorous clean-up procedure, such as that of de Faubert Maunder *et al.*[5], which includes a dimethylformamide–hexane partition and passage through a column of prepared alumina. Because these unknown compounds are similar in nature and properties to the organochlorine pesticides, they also pass through this and similar clean-up procedures; consequently, they

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often interfere seriously with determinations of *pp'*-TDE and *pp'*-DDT in wildlife because of overlapping of the chromatographic peaks. Suitable procedures have been devised to overcome this difficulty of interfering peaks. One method is to effect a better resolution of these particular peaks by choice of a suitable stationary phase for GLC[4]. Another method, which also demonstrates the presence of these compounds in the sample, is the use of a preliminary thin-layer chromatographic treatment[7]. The cleaned-up extracts are examined on silica gel chromatoplates using a 1 per cent solution of acetone in hexane as mobile phase[8]. Spots corresponding to the compounds can then be found at R_F values between 0·8 and 1·0, whereas the commonly occurring organochlorine pesticides and their metabolites produce spots of lower R_F values. A similar separation can also be shown with reverse phase paper chromatography[9] whereby the unknown compounds move only a very short distance from the base line compared with the pesticides and their metabolites.

Gas-liquid chromatography using dual channel detection systems with separate electron-capture and flame-ionization detectors has also been used to demonstrate the presence of large amounts of weakly electron-capturing material, in addition to known pesticide residues, in the eggs of oyster catchers (*Haematopus ostralegus*)[9].

Experience in this laboratory has shown that these compounds occur most frequently and in the largest proportions in the livers, fat and eggs of birds, particularly terrestrial predators such as sparrowhawks (*Accipiter nisus*) and kestrels (*Falco tinnunculus*) and marine feeders such as guillemots (*Uria aalge*) and kittiwakes (*Rissa tridactyla*); they also commonly occur in freshwater fish. We have detected small amounts in human and animal fat samples, but less frequently and in much smaller proportions than in avian samples.

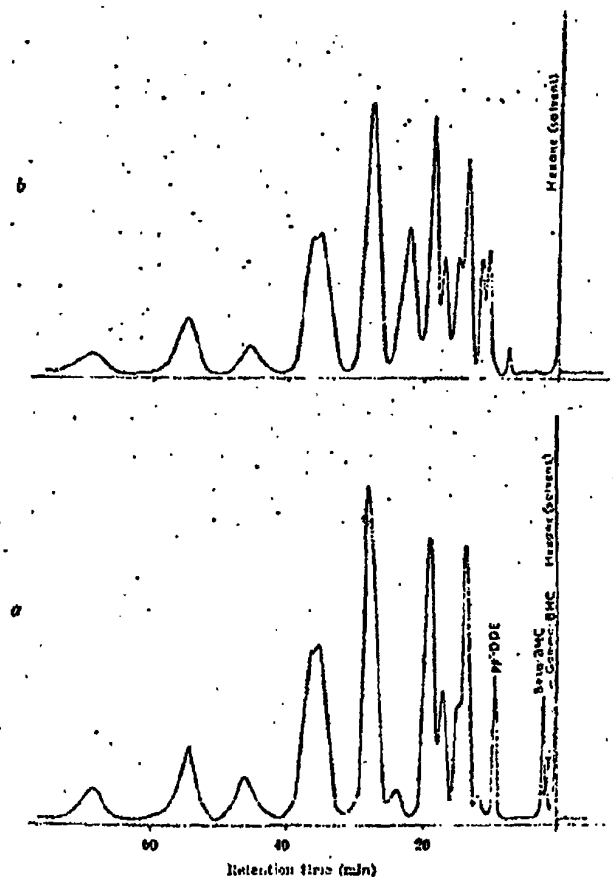

Fig. 1. Gas-liquid chromatograms of (*a*) extract of kestrel liver and (*b*) a commercial polychlorobiphenyl resin.

A similar series of chromatographic peaks has been observed by Holden[10,11] when examining extracts of seals and fish taken in Scottish waters. He found that the peaks given by the extracts of fresh and sea-water fish represented fairly low concentrations, but those given by extracts of seal blubber corresponded to much larger proportions, of the order found by this laboratory in birds' eggs.

Despite considerable curiosity about the identity of these compounds, little progress has been made in identifying them, but there has long been a general supposition that they were either further breakdown or condensation products of the organochlorine pesticides or possibly loose compounds of these with natural products such as protein matter.

Jensen, however, succeeded in identifying a series of such peaks as corresponding to polychlorobiphenyl compounds, in pike taken in different parts of Sweden, and in an eagle[12]. We have now been able to show that the long retention time compounds occurring in British wildlife are also polychlorobiphenyl compounds.

Polychlorinated biphenyl has been in common commercial use for some time as a plasticizer in paints, resins and plastics. It also has electrical insulating properties and can be used for a number of other protective purposes. Commercial preparations of this material are usually graded according to the degree of chlorination, but all of them are substantially complex mixtures of polychlorobiphenyl compounds that could be expected to resemble the organochlorine pesticides in their chemical and physical properties. In the prescribed extraction and GLC conditions, these mixtures produce a series of chromatographic peaks of the type in question.

The compounds occurring in British wildlife correspond to the more highly chlorinated biphenyl compounds rather than the more highly chlorinated types. These highly chlorinated biphenyls also occur to some extent in the analogous polychloroterphenyl preparations which have commercial uses similar to those of polychlorobiphenyl. It is possible that some of the compounds with very long retention times occurring in British wildlife are highly chlorinated polychloroterphenyl compounds.

Fig. 1*a* shows the gas-liquid chromatogram of an extract from a kestrel liver. The peaks corresponding to *pp'*-DDE (0·13 ng), beta-BHC (0·33 ng) and gamma-BHC (0·03 ng) are labelled. The other peaks form the series referred to above and until now would have been regarded simply as "unknown organochlorine compounds not corresponding to any known pesticide or its metabolites". Fig. 1*b* shows the analogous chromatogram of a commercial polychlorobiphenyl resin. So far as retention times are concerned and apart from the pesticide peaks, there is exact matching for all but a few peaks in the two chromatograms.

The chromatograms shown were obtained by use of a silicone gum (*GE-SE 52*) column, but this precise correspondence between the retention times of the peaks for the unknown compounds and those from the polychlorobiphenyl resin has also been demonstrated on 'Apiezon *L*'

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Table 1. RELATIVE RETENTION TIMES ON THREE DIFFERENT GLC COLUMNS (Excluding lines for known pesticides)

Silicone *GE-SE 30* column (heptachlor = 1·00)		Apiezon *L* column (heptachlor = 1·00)		Cyanosilicone *GE-XE 60* column (heptachlor = 1·00)	
KLE	PCB	KLE	PCB	KLE	PCB
1·42	1·42	2·31	2·30	1·21	1·21
1·53	1·52	2·67	2·67	1·34	1·33
1·91	1·90	3·01	3·05	2·20	2·19
2·81	2·81	5·06	5·06	2·62	2·80
2·89	2·84	5·09	5·05	2·67	2·92
3·71	3·70	6·57	6·55	3·60	3·60
4·68	4·64			4·03	4·03
5·02	5·02			5·11	5·11
7·10	7·11				

KLE, Kestrel liver extract; PCB, polychlorobiphenyl resin.

and cyanosilicone columns. The actual retention times recorded for the peaks on these three types of column are given in Table 1.

Further confirmation as to the identity of these compounds has been obtained by a study of their behaviour on thin-layer chromatoplates, alumina and silica gel absorption columns and reverse phase paper chromatograms. In addition, these compounds have been shown to resemble the polychlorobiphenyl compounds in their chemical inertness. Thus they are not easily modified by simple chemical reactions to produce readily identifiable shifts in their retention times on GLC as can be done, for instance, to *pp'*-DDT by hydrolysing it to *pp'*-DDE or to aldrin by oxidizing it to dieldrin.

A number of livers and eggs taken from birds in the British Isles or their coastal waters have been examined in this laboratory with results similar to those obtained for the kestrel liver referred to earlier. While the identification of these compounds in British wildlife may solve the mystery of these peaks on GLC chromatograms, it raises, however, many other questions. Why, for example, do these compounds seem to be accumulated, most frequently and in the largest proportions, in certain types of wildlife such as birds, seals and fish, and only at the most in very small proportions in man or his domestic animals such as cows or sheep? In this respect they seem to differ markedly from the organochlorine pesticides which seem to have invaded nearly all aspects of our environment and to which they bear a strong resemblance chemically and physically.

Some of the samples of birds examined in this laboratory have seemed to contain higher proportions of polychlorobiphenyl compounds than organochlorine pesticides. For example, the peaks on the chromatogram for the kestrel liver shown in Fig. 1 were estimated to be equivalent to about 12 ng of polychlorobiphenyl compounds. This must be contrasted against the organochlorine pesticide residues listed above. These total only about 0·8 ng of which 0·33 ng is beta-BHC, an isomer of BHC which is generally regarded as non-toxic to wildlife.

Great concern has often been expressed by conservationists about the effects of pesticides on wildlife, particularly birds. The identification of these other organochlorine compounds in wildlife prompts enquiries as to the possible toxic effects they may be having. That these compounds are toxic is generally agreed[10,11]; the polychlorobiphenyls are in fact regarded as an industrial hazard and threshold limits for them in air have been advised[11].

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1 Roburn, J., *Analyst*, **90**, 467 (1965).
2 Harrison, R. B., *J. Sci. Food Agric.*, **17**, 70 (1966).
3 *Report of the Government Chemist*, 79 (HM Stationery Office, London, 1963).
4 Walker, C. H., Hamilton, G. A., and Harrison, R. B., *J. Sci. Food Agric.*, **18**, 123 (1967).
5 de Faubert Maunder, M. J., Egan, H., Godly, E. W., Hammond, E. W., Roburn, J., and Thomson, J., *Analyst*, **89**, 168 (1964).
6 Shannon, J. H., and Tatton, J. O'G., *J. Chromatog.*, **27**, 253 (1967).
7 Abbott, D. C., Egan, H., and Thomson, J., *J. Chromatog.*, **16**, 451 (1964).
8 Evans, H., *Analyst*, **87**, 569 (1962).
9 *Report of the Government Chemist*, 102 (HM Stationery Office, London, 1966).
10 Holden, A. V., and Marsden, K., *J. Proc. Inst. Sew. Purif.*, 223 (1966).
11 Holden, A. V., *J. Appl. Ecol.*, **3**, suppl., 45 (1966).
12 Jensen, S., *New Sci.*, **32**, 612 (1966).
13 Sax, N. Irving, *Dangerous Properties of Industrial Materials*, 147 (Reinhold Publishing Corporation, New York, 1963).
14 Brown, H. M., *Chemist-Analyst*, **55**, 53 (1967).
15 *Documentation of Threshold Limit Values*, 41 (Committee on Threshold Limit Values, 1966).

Quasi-stellar Sources, Supernovae and Novae

by
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By considering the opacity of a surface layer of a star, the author concludes that quasi-stellar sources, supernovae and novae may be different manifestations of the same mechanism.

It is usually considered that the outbursts of novae and supernovae are fundamentally different processes, but I shall here propose a mechanism which can account for both phenomena, and which at the same time can account for other problems concerned with quasi-stellar sources (quasars). In particular, I suggest that these outbursts are likely to be anisotropic (as indeed they are observed to be for novae). Because the energy released during a supernova outburst is comparable with the stellar rest energy, an anisotropic outburst could mean that the star is accelerated to relativistic velocity in order to conserve momentum, thus affording a Doppler explanation of the large red-shifts of the quasi-stellar sources.

Consider the opacity of a layer, L, at the surface of a star. Let ΔR be the thickness and R the radius of L, and let L contain plasma at temperature, T, with electron and positive ion densities N_e and N_i respectively. L will be opaque to radiation of wavelength λ provided that τ is about 1, where τ is the optical depth of L. A major contribution to τ is τ_{ff}, the optical thickness due to free-free absorptions. It is known that $\tau_{ff} \propto \lambda^2 T^{-3/2} N_e N_i \Delta R$ (ref. 1), where induced emissions are included. Suppose, now, that L is compressed or dilated without changing the total numbers of ions; that is, ΔR is varied under conditions which maintain constant $4\pi R^2 \Delta R N_i$, so that $\Delta R N_i =$ constant. Evidently $\tau_{ff} \propto N_e N_i \Delta R \propto N_i \propto 1/\Delta R$. Thus compression of L increases its opacity, while dilation reduces the opacity.

Of course, the total number of ions will not remain constant in practice, because the recombination rate is proportional to $N_e N_i$. If the percentage of atoms ionized is large, the density of unionized atoms, N_u, will therefore be proportional to $N_e N_i$. This means that the contribution to the opacity made by bound-bound and bound-free transitions within unionized atoms is also proportional to $N_e N_i \Delta R$. This effect, recognized to be important for stellar pulsation[12], further increases the opacity of L on compression.

Some consequences of this variable surface opacity will now be examined. Under equilibrium conditions the

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