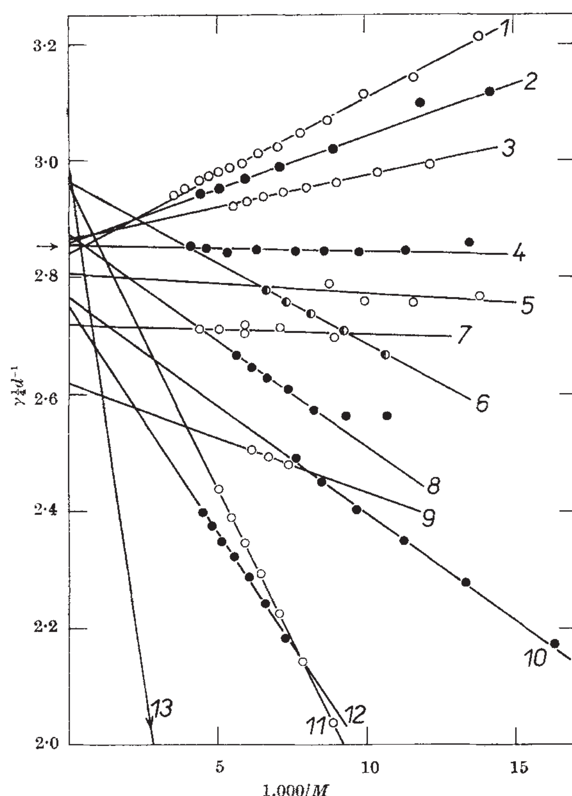

Fig. 1. The statistically correct examination of the additivity of the parachor by plotting $\gamma^{1/4}d^{-1}$ versus $1/M$ according to equation (2). The individual lines represent the following homologous series: 1, *n*-alkanes; 2, alkenes-1; 3, alkines-1; 4, ethers; 5, aldehydes; 6, α,ω-dinitriles; 7, ketazines; 8, 4-alkylpyridines; 9, alkyl benzyl ketones; 10, 1-nitro compounds, 11, α,ω-dichlorides; 12, alkyl nicotinates; 13, α,ω-dibromides. The arrow denotes the point of intersection claimed for the value 40·0 of the CH_2-increment. The majority of experimental values were taken from ref. 3

$$\gamma^{1/4}D^{-1} = \frac{C}{M} + \frac{P(CH_2)}{14,026} \quad (2)$$

where C is a constant characteristic for each homologue series and $P(CH_2)$ is the increment of the methylene group. From a graphical representation (Fig. 1), it is seen that equation (2) is not fulfilled even for homologue series of relatively simple derivatives. Since the straight lines do not intersect in one point, the increment of the group CH_2 cannot be determined common for all homologue series so that contentions concerning its right value[1-3] are ungrounded. It follows that the generally used formula, equation (1), is not well founded; the additivity is only approximately pretended by the additivity of the molecular weight or of the molar volume.

From both measured quantities the density exerts a far greater influence on the result. The influence of the surface tension, weakened still by the empirical exponent 1/4, represents only a smaller correction which does not contribute to the additive character of the over-all expression and, on the contrary, causes deviations from the additive behaviour; for example, at least with simpler hydrocarbon derivatives the principle of additivity is fulfilled more exactly for the molar volume than for the parachor. Now since measured values of the surface tension have been used almost exclusively for calculation of the parachor and discussed in this form, very little is known about the dependence of the surface tension on the structure.

Both quantities under consideration are markedly statistically dependent (substances with a greater specific weight have a higher surface tension) and their influence on the parachor is mutually compensated. Therefore, in the unfavourable case, up to 1/4 of all organic compounds can yield experimental values acceptable for a chosen structure; at the same time especially the surface tension can achieve even more than one half of the possible values, an acceptable accuracy of the result being maintained.

Conclusions concerning the structure, made on the basis of the parachor, are therefore dubious. Some authors[2,3] attempt to improve this method by a detailed specialization of the increments and by introducing corrections for interaction of the groups. This leads to a certain arbitrariness on one hand, and to a successive loss of the additive character on the other. In a similar way, the dependence of every physical quantity on structure can be grasped; instead of the parachor it would be more simple and suitable to discuss in this way the molar volume.

It can be concluded that the conception of the parachor has no material significance and its liquidation can be suggested.

O. Exner

Polarographic Institute,
Czechoslovak Academy of Sciences,
Prague.

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Free Radicals formed during the Oxidation and Reduction of Peroxides

The reduction of hydrogen peroxide by transition metal ions is thought to involve hydroxyl radicals as intermediates[1]. We have found that dilute solutions of titanous ion and hydrogen peroxide (about 10^{-2} M) react together to form a free radical of short life. This has been observed by using a flow system in which the reactants are mixed just before entering the cavity of a Varian electron spin resonance spectrometer. The spectrum is a single line near $g = 2$, width about 3 gauss, the intensity of which at maximum flow-rate is proportional to $[Ti^{3+}]$ and $[H_2O_2]$ when these are low.

The radical may be $\cdot OH$ or $\cdot O_2H$, according to the reactions[1]:

$$Ti^{3+} + H_2O_2 \rightarrow Ti^{4+} + OH^- + \cdot OH$$
$$OH + H_2O_2 \rightarrow H_2O + \cdot O_2H$$

There is evidence, however, that the perhydroxyl radical gives a much broader signal because, when hydrogen peroxide[2], *t*-butyl hydroperoxide, and cumyl hydroperoxide are oxidized by ceric ion, single lines of width 27, 14, and 6 gauss, respectively, are observed, presumably corresponding to peroxy radicals:

$$Ce^{4+} + RO_2H \rightarrow Ce^{3+} + H^+ + RO_2^{\cdot}$$

An attempt to observe the *t*-butoxy radical in the reaction between *t*-butyl hydroperoxide and titanous ion resulted in the appearance of a quartet of intensities 1 : 3 : 3 : 1, the distance between adjacent peaks being about 24 gauss. This is apparently due to the methyl radical[3] arising from the reactions:

$$Ti^{3+} + Me_3COOH \rightarrow Ti^{4+} + OH^- + Me_3CO$$
$$Me_3CO\cdot \rightarrow Me_2CO + CH_3\cdot$$

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These results suggest that the radical observed in the reaction between hydrogen peroxide and titanous ion is hydroxyl.

The signal corresponding to this radical disappears when the reactant solutions contain various aliphatic or aromatic compounds and is replaced by spectra of new radical intermediates. For example, 2-propanol gives a septet together with a weaker quartet, peak-to-peak distances being about 20 and 24 gauss, respectively. These are similar to spectra previously ascribed to Me_2C–OH and CH_3 which were observed in the solid state at low temperatures[3,4] except that the hyperfine line-widths which we observe are narrower (approximately 1·5 and 2·5 gauss, respectively).

These results confirm that the hydroxyl radical is very reactive, may be obtained in aqueous solution at room temperature, and can be used to generate other free radicals. A discussion of all the spectra and a description of the flow system will be published.

W. T. DIXON
R. O. C. NORMAN

Dyson Perrins Laboratory,
University of Oxford.

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BIOCHEMISTRY

An Examination of HeLa Cancer Cells for Production of Gamma-Globulin

A METHOD has been described for detecting and measuring the autonomous production of well-defined proteins by cells in tissue culture[1,2]. The method is exceedingly sensitive and is applicable even when the cells are cultivated in a 'natural' nutrient medium, that is, a medium also containing serum; such media have an enormous excess of pre-existing proteins. The tissue is incubated in presence of a radioactive amino-acid and the whole of the soluble protein is extracted. Thereafter individual proteins are isolated from the mixture and burnt, and the carbon dioxide obtained tested for radioactivity with a gas counter (efficiency nearly 100 per cent)[3,4]. The identity of a fraction can be confirmed by treatment with specific antiserum, followed by determination of the distribution of the radiocarbon between precipitate and supernatant. Blank experiments show that after incubation of natural medium with radioactive amino-acid without cells practically no radiocarbon is found in protein.

Now we have tested HeLa human cervix carcinoma cells, grown as described before[5], for the production of γ-globulin. Pieces of cultivated HeLa tissue were trypsinized, and aliquots of the suspension obtained were introduced into plasma-coated roller tubes. The resulting monolayers were incubated several days at 37° C with a natural medium. Then the medium was changed for a protein-free medium of exactly known composition ('synthetic' medium[6,7]), also containing 0·5 μc. of 1-^{14}C-D,L-leucine per tube. The incubation was continued for 4 days, 1-ml. human cord serum was added as a carrier to each roller tube, the cells were broken by repeated freezing and thawing and the cell debris removed by centrifugation. The supernatant was freed from low-molecular substances, including the radioleucine, by ultra-filtration under pressure, followed by repeated washing with physiological saline solution[2].

Table 1. RADIOACTIVITY IN THE γ-GLOBULIN FRACTION FROM HELA CELLS

Exp. No.	Final cell No.	Activity of total soluble protein (d.p.m.)	Activity of γ-globulin (d.p.m.)	Part of protein activity in γ-globulin (per cent)
1	352,000	72,000	1,330	1·8
2	352,000	72,000	1,940	2·7
3	429,000	84,000	2,340	2·8
4	429,000	84,000	2,980	3·5

Thereafter, the γ-globulin was separated from the mixture of the soluble proteins by precipitation in the cold with ethanol[8]. Between 3·6 and 5·6 mg γ-globulin—of course, mostly from the carrier—were recovered. The purity of the preparation was checked by electrophoresis in starch gel[9] and staining, and no protein found elsewhere than in the γ-globulin band. The activities per roller tube found in four separate experiments and calculated to the total amount of γ-globulin present in the carrier are given in Table 1. The cell numbers were obtained from counts, after trypsinization, of the cells in parallel tubes, that is, in roller tubes which had been charged with equal numbers of cells and afterwards kept in the same conditions as the tubes used for radiochemical analysis.

In further experiments the soluble proteins obtained by ultra-filtration were subjected to electrophoreses in agar gel. In each case, two parallel samples (0·2 ml. of 7 per cent protein solution—the protein again mostly derived from the serum carrier) were used. After electrophoresis, one sample was stained to indicate the position of the protein bands. The other sample was cut in strips at right angles to the field, the strips were burnt, and the radioactivities per unit strip width determined. Only 3·3 per cent of the total radiocarbon found in the electropherogram was contained in the γ-globulin fraction, in reasonable agreement with the results given in Table 1. The percentage is, however, less than that given previously[1,2]. Essentially the same result (3·7 per cent) was obtained after incubation with radioleucine in a natural instead of a synthetic medium; in this case no protein carrier was added after incubation. Incidentally, it was confirmed in the last-mentioned experiments that the protein of the medium remains practically non-radioactive on incubation with active amino-acid in the absence of cells.

Finally, a γ-globulin preparation obtained from HeLa cells incubated with radioleucine by the ethanol fractionation method and afterwards purified by electrophoresis in starch gel was tested radio-immunologically. 1·2 mg γ-globulin were mixed in the optimal ratio with antiserum against human γ-globulin from rabbits. The antiserum had been tested for specificity by the Ouchterlony method[10]. The total volume was 6 ml. The precipitate formed and the supernatant were burnt separately, and the radioactivities measured. The activity of the precipitate was found to be ($1·2 \pm 1·9$) disintegrations per minute (d.p.m.), that of the supernatant (270 ± 10) d.p.m. Thus the protein fraction from HeLa cells which behaves in respect to precipitation with alcohol as well as in respect to electrophoresis in starch like γ-globulin cannot be identified as normal human γ-globulin by immunology.

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