influenced by X-radiation causing the appearance of aberrant serum proteins.

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[1] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[2] Crowle, A. J., *Immunodiffusion* (Academic Press, London, 1961).
[3] Jordan, R. T., Banovitz, J., Trapani, I. L., and Campbell, D. H., *J. Immunol.*, **88**, 314 (1962).
[4] Rittenberg, M. B., and Nelson, E. L., *Fed. Proc.*, **19**, 198 (1960).
[5] Rittenberg, M. B., and Nelson, E. L., *Amer. Nat.*, **96**, 327 (1960).

A Simple Method for detecting Single Antibody-forming Cells

To my knowledge no method is available for determining the number of antibody-forming cells by simple counting in a hæmocytometer. For this reason the technique proposed here provides possibilities not yet covered by the already known methods for the detection of single antibody-forming cells[1–3].

Mice were immunized against sheep red cells by intraperitoneal injection of 10^9 cells twice a week, for three consecutive weeks. Six days after the last injection the spleen was removed and 10^7 washed spleen cells were suspended in a test-tube in 1 ml. of a tissue culture medium[4], containing 1 per cent washed sheep red cells. The presence of calf serum in the medium was shown to be not essential. The test-tubes were incubated during 2 h at 37° C in a roller tube apparatus. Thereafter the cells were pipetted into a hæmocytometer and counted. It was observed that a number of nucleated cells was more or less densely surrounded by agglutinated sheep cells, which was ascribed to the action of the agglutinins produced by the spleen cells (Table 1). The number of agglutinated erythrocytes clustered around a cell is supposed to reflect the amount of antibody produced by that cell. Although comparison with the results of Jerne[2] is rather difficult in view of the fact that another animal species and a different immunization technique was used, I got the impression that in my system a higher yield of antibody-forming cells is obtained. With 0·01 molar potassium cyanide in the culture medium Jerne[2] was able to stop cellular antibody formation. Addition of this concentration to our test-tubes before incubation reduced the number of clusters to about a tenth as compared with the non-treated suspensions. No cluster formation was seen in spleen cell suspensions from non-immunized animals or from animals that had been immunized with φX174 phage. Thymus cells obtained from immunized animals did not show any cluster formation, which is in accordance with the present opinion about the antibody-forming capacity of the thymus[5,6].

Of the numerous applications for this method some obvious experiments are suggested; for example, the number of antibody-forming cells in the lymphoid tissues after different routes of immunization may be determined quantitatively. The problem whether a single cell from an animal immunized against two different antigens produces two different antibodies may be approached by using a mixture of sheep and fowl erythrocytes. The different morphology of these cells makes it possible to decide whether the cluster around a single cell consists of a mixture of the two red cell types, indicating the formation of two types of antibodies by a single cell.

Here the use of red cells coated with various antigens is now being investigated.

Table 1. NO. OF ANTIBODY-FORMING CELLS IN A SUSPENSION OF TEN MILLION NUCLEATED CELLS

Mouse No.	Immunized with	Source of cells incubated	No. of nucleated cells with clusters counted in hæmocytometer	Calculated No. of antibody-forming cells per 10^7 nucleated cells
1	Sheep red cells	Spleen	24	4×10^4
2	Sheep red cells	Spleen	17	3×10^4
3	Sheep red cells	Spleen	36	6×10^4
			3*	5×10^3
		Thymus	0	
4	Phage φX174	Spleen	0	
5	—	Spleen	0	

* 0·01 mol. KCN added before incubation.

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[1] Nossal, G. J. V., and Lederberg, J., *Nature*, **181**, 1419 (1958).
[2] Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).
[3] Vazquez, J. J., *Lab. Invest.*, **10**, 1110 (1961).
[4] Fisher, G. A., *Ann. N.Y. Acad. Sci.*, **76**, 673 (1958).
[5] Thorbecke, G. J., and Keuning, F. J., *J. Immunol.*, **70**, 129 (1953).
[6] Marshall, A. M. E., and White, R. G., *Brit. J. Exp. Path.*, **42**, 379 (1961).

RADIOBIOLOGY

Porphine-like Substances: Probable Synthesis during Chemical Evolution

IN the literature[1–4] most of the authors dealing with the problems of origin of life are convinced that, at some stage of chemical evolution, porphyrins must have developed. However, until recently there was no laboratory evidence that porphine-like substances could be synthesized non-enzymatically from simple precursors. In a previous paper[5] it was shown that traces of porphine-like substance, specifically α,β,γ,δ-tetraphenylporphine, was formed in a mixture of pyrrole and benzaldehyde in pyridine irradiated with cobalt-60 γ-radiation. In the experiments recorded here[6] an aqueous suspension of pyrrole and benzaldehyde was irradiated at room temperature with either ultra-violet or visible light. The distance in both cases was 25 cm from the light source. For ultra-violet radiation a 100-W Hanovia Utility model lamp was used. The radiation transmitted through the filter was 45 per cent at 2500 Å and 90 per cent at 3000 Å and the output of the lamp was $1{\cdot}5 \times 10^{15}$ quanta/sec/cm² or 5×10^{16} quanta/sec/area of the mixture exposed toward the radiation. The visible light source was a 100-W tungsten filament lamp bulb. In addition an aqueous suspension and a solution in pyridine of pyrrole and benzaldehyde were placed in semi-darkness without irradiation and then analysed for the products.

In all cases a porphine-like product, that is, α,β,γ,δ-tetraphenylporphine, was obtained. The final separation of products obtained from either irradiated or non-irradiated mixtures and/or suspensions was achieved by column chromatography, using activated alumina and 'Florosil' as adsorbents and chloroform as the eluent. The identity of the product was established by comparison of the visible spectra of the base, acid salt and zinc chelate of the product with the spectra of compounds synthesized by the method of Ball, Dorough and Calvin[7]. For quantitative estimation of the yield a molar extinction coefficient of $18{\cdot}7 \times 10^3$ at 515 mμ maximum for the free base was used, as suggested by Thomas and Martell[8].

If, instead of water, pyridine was used as a solvent in the system, and the mixture was then irradiated for 2 h with the 2537 Å mercury line, or left without irradiation, no porphine-like substances could be detected by previously used methods.

Figs. 1 and 2 represent the total yields of porphines isolated from mixtures stored in chloroform, plotted against time. Each line in the graph represents different irradiation conditions.

The results of this work indicate that the presence of water, or rather the suspension of the organic matter in water, increases the yield of porphine-like substances con-