INCORPORATION OF LABELLED AMINO-ACIDS IN ANTIBODIES SYNTHESIZED IN VITRO BY CELLS OF IMMUNIZED RABBITS.

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The appearance of radioactive protein in the medium of a tissue culture incubated with an isotopically labelled amino acid is an indication that protein has been synthesized de novo and not merely released, if it is believed that an exchange reaction does not occur. Such evidence for de novo synthesis of antibody (Ab) has been provided by Mountain (1955) and others in studies with tissue fragments from immunized rabbits and by Vaughan et al (1960) who used isolated cells. In these experiments soluble protein antigens were studied and the yield of radioactive antibody was so little as to require the use of a non-isotopic homologous precipitate as carrier.

We have succeeded in avoiding this step by employing a particulate antigen, sheep erythrocytes (S.E.), to immunize the rabbits and to remove the radioactive Ab formed in the tissue culture fluid. Adult rabbits were immunized by 9 intradermal injections of 0.1 ml of S.E., administered over a period of 2 weeks into the left foot pad. Seven to 21 days after immunization a booster dose of 0.1 ml was given in the same fashion. Three days later the animals were bled and the popliteal lymph nodes were excised from the injected and contralateral extremities. Lymph node cells were obtained by gently teasing the nodes in Eagle's medium and passing the debris through a nylon sieve. The cells were centrifuged at 600 RPM for

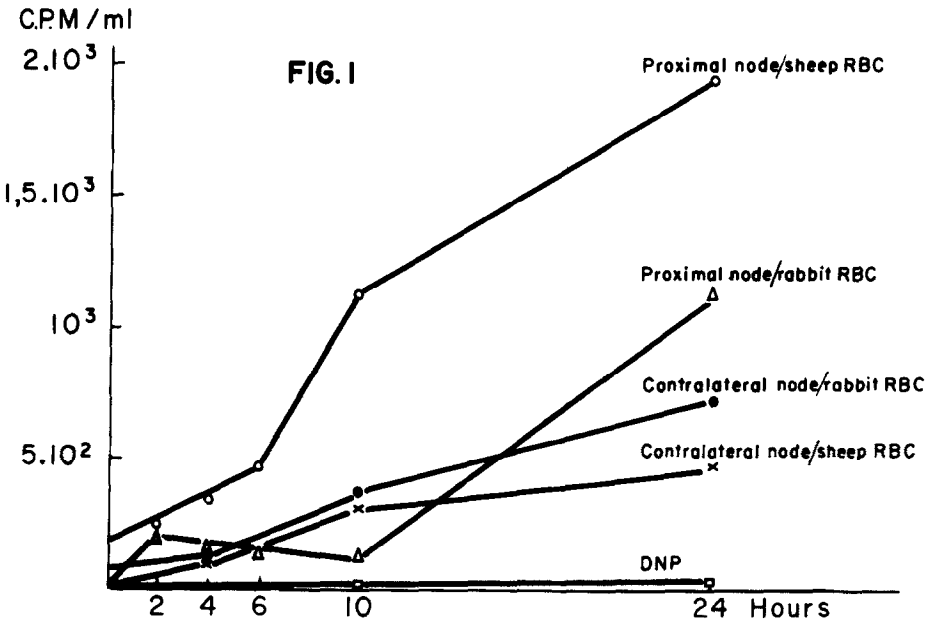
10 minutes and washed twice. The cells were suspended in a sufficient amount of Eagle's medium to give a concentration of 10^7 cells/ml and 20 ml aliquots of the cell suspension were distributed in 150 ml Erlenmeyer flasks. In these experiments radioactive valine with a specific radioactivity of 74 mC/mM and in a final amount of 1 μC/ml was used. In each experiment four separate flasks were employed with 1) cells from the proximal lymph node, 2) cells from the contralateral lymph node, 3) cells from the proximal node with dinitrophenol added (10^{-2} M), and 4) cells from non-immunized rabbits. In an additional control, the radioactive valine was added at the end of the experiment to the cells from the proximal lymph node. The flasks were aerated with a mixture of 95% air-5% CO_2 and gently shaken at 37^o C.

Samples were taken at intervals for determination of cell viability (staining by Trypan blue), total protein (by trichloroacetic acid precipitation) and Ab radioactivities. The latter measurement was made by shaking 1 ml of S.E. (with 3×10^8 cells) with 0.25 ml of the culture medium and 0.75 ml of saline containing non-radioactive valine (10^{-2} M), for 1 hour at 37^o C. This amount of S. E. was found, in preliminary studies, to represent an excess of antigen. The S. E. were separated on millipore filters (saturated by previous soaking in 10^{-2} M valine), and the filters were extensively washed with valine in saline, dried and counted on a thin window Geiger counter (28% counting efficiency).

Under such conditions we have observed in vitro synthesis of anti S.E. antibodies by cells of rabbits after a secondary stimulation. A typical experiment is shown in Figure I. It can be seen that Ab synthesis by the locally stimulated cells (proximal node) proceeds at a faster rate than Ab synthesis by the cells from the contralateral node. However, both systems synthesized Ab during the 24 hours of incubation. At the end of this period, only 30% of the cells were viable. A proof that the radioactive substance fixed on the S.E. was γ-globulin was provided by the fact that previous treatment of the culture samples by an excess of sheep anti-rabbit γ-globulin

followed by the removal of the precipitate, before addition of S.E., reduced the radioactivity bound on the erythrocytes to 15% of its original value.

To determine the specificity of Ab attachment, the erythrocytes (R.E.) from the same rabbit from which the lymph node cells were taken were used as a control. Surprisingly these autologous R.E. were found to fix radioactivity from the tissue culture fluid samples, although to a lesser degree than S.E. (Fig. I). Previous heating of the samples at 56° C for 30 minutes did not affect this fixation but it was greatly reduced by specific precipitation of rabbit γ-globulin by sheep immune serum. The possibility that these antibodies are true auto-antibodies requires more study.



If the ratio of radioactive Ab to radioactivity of total protein is computed (Fig. II), it can be seen that highly stimulated cells are able to produce a high proportion of their protein output in specific Ab, i.e., 30 to 40%. From these data it can be calculated that 100 to 300 μg of Ab are synthesized per gram of cells, per 24 hours. These values are consistent with the data of Vaughan et al (1960) and of Stavitsky (1958).

We believe that the system described provides a sensitive and reliable method of studying the synthesis of antibody in vitro.

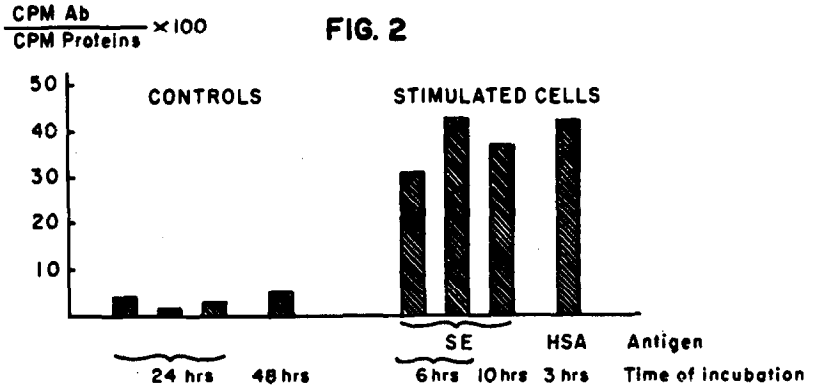


FIG. 2

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