arthritic patient. In both experiments the difference between the means is significant by Student's $t$ test ($P < 0.005$), suggesting a decreased reactivity of lymphocytes from rheumatoid-arthritic patients to phytohaemagglutinin compared with healthy controls.

Preliminary experiments with control lymphocytes stimulated with protein A indicated that the optimum concentration of this mitogen was $10 \mu{g}$/ml. The results shown in Table 1 suggest that the optimum incubation period is 4 or 5 days. Table 2 shows the response of lymphocytes from controls and rheumatoid-arthritic patients to various concentrations of protein A when cultured for 4 days. The results of two trials are shown and a different control/patient pair was used in each case. As shown in Table 2, optimal stimulation occurs at a protein A concentration of $10 \mu{g}$/ml with cells from controls and rheumatoid-arthritic patients. With concentrations of $80 \mu{g}$/ml or more the stimulation is only slight and probably not significant. However, over the range 5–50 $\mu{g}$ of protein A/ml the stimulation of lymphocytes from rheumatoid-arthritic patients is decreased compared with control cells. At the optimum concentration ($10 \mu{g}$/ml), the difference between the means is significant in both trials ($P < 0.01$).

The decreased mitogenic response of lymphocytes from rheumatoid-arthritic patients to phytohaemagglutinin suggests a possible defect in T-lymphocyte function, as has been previously suggested by other workers (Horwitz & Garrett, 1977). The response of cells from rheumatoid-arthritic patients to protein A suggests that B-lymphocyte function might also be impaired, despite the observed increase in immunoglobulin-rheumatoid-factor synthesis in such cases.

Preliminary experiments following the incorporation of $[^3H]$leucine in protein A-stimulated lymphocyte cultures suggest that a similar response pattern will emerge for both DNA and protein synthesis.


Inhibition of Capping of Surface Immunoglobulins at Femtomolar Concentrations of Adriamycin, Compound ICRF-159 and Tetrodotoxin

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We have reported previously that the anti-tumour antibiotic adriamycin inhibits the activity of native cardiac $(Na^+ + K^+)$-ATPase* at the incredibly low concentration of $10^{-15}$M (Gosalvez & Blanco, 1975). This inhibition is related to the chelating abilities of adriamycin, because the inhibition of the $(Na^+ + K^+)$-ATPase and ion transport is counteracted by $Ca^{2+}$ (van Rossum & Gosalvez, 1976) and a metallic derivative of adriamycin made by chelation with ferric iron was not active in the ATPase (Gosalvez, 1976).

We now report that adriamycin and two other potent chelating agents for ferric iron, compound ICRF-159 and tetrodotoxin, inhibit the capping of surface immunoglobulins at femtomolar $(10^{-15}$M) concentrations. Compound ICRF-159 is a derivative of EDTA with strong anti-tumour activity (Hellman et al., 1969) and tetrodotoxin is an inhibitor of Na* conductance in nerve (Hille, 1968). The effect of these compounds in capping, a process well known to depend on cell membrane microfilaments (Schreiner et al., 1977),

* Abbreviation: $(Na^+ + K^+)$-ATPase, sodium-plus-potassium ion-dependent adenosine triphosphatase.
Fig. 1. Effects of increasing concentrations of adriamycin (●), tetrodotoxin (▲) or compound ICRF-159 (■) on the capping of different cell preparations

Adriamycin and tetrodotoxin inhibit 50% of capping at 10^{-15} M and compound ICRF-159 inhibits 25% at 10^{-15} M.

permits us to speculate that its effect on the (Na^+ + K^+)-ATPase and ion transport could be mediated through a system of microfilaments.

The capping of surface immunoglobulins was measured by fluorescence microscopy by using lymphocytes from the spleen of Swiss mice. Samples of 1 × 10^6 lymphocytes suspended in 50 μl of phosphate-buffered saline were mixed with 50 μl of fluorescein-treated goat antiserum to mouse IgG-globulin (2.2 mg/ml antibody concentration, Hyland) and 10 μl of phosphate-buffered saline containing the amount of drug desired. The mixture was incubated without agitation at 0°C for 40 min, and then the cells were washed three times with phosphate-buffered saline. The cells were resuspended from the last centrifugation in 0.1 ml of medium 199 (Difco) and were then incubated for 45 min at 37°C without agitation to perform the capping. At the end of the warm incubation period, the tubes with the cells were immersed in ice and samples were obtained for observation under fluorescence microscopy. Two observers counted the cells in several fields noting the total number of cells per field, the total number of stained fluorescent cells per field and the number of cells in which the fluorescence was mainly distributed in a cap at one pole of the cells. Capping was expressed as the percentage of cells with caps within the total of stained cells; the average of the values of the two investigators was used. In our experiments, cell capping in 45 min varied between 60 and 80%. Two duplicate tubes were made for each concentration of drug tested. Controls were tubes in which the drug was omitted from the cold incubation.

Fig. 1 shows the effect of increasing concentrations of adriamycin (●), tetrodotoxin (▲) or compound ICRF-159 (■) on the capping of different cell preparations. The extent of capping is expressed as the percentage of control values in the absence of the drug. When the drug was diluted to 10^{-22} M, which is when there was no drug in the tubes, the capping was 100% of the control; however, at concentrations of 10^{-20} M and above, there is a progressive inhibition of capping with the three drugs along a wide range of concentrations. At 10^{-15} M, the inhibition of capping by adriamycin and tetrotoxin is about 50%, and by compound ICRF-159, is about 25%. To obtain inhibition at such incredibly low concentrations, it is crucial to make the warm incubation (to perform the capping), without agitation, at 37°C and with a duration of at least 46 min. It is sufficient to lower the temperature to 30°C to decrease the duration of incubation to 30 min or to incubate with agitation to make the inhibition of capping at femtomolar concentrations dis-
Fig. 2. Inhibition of capping by mixing the cells with three-times washed-adriamycin (0.1 μM)-treated cells

Effect of increasing concentrations of treated cells is shown.