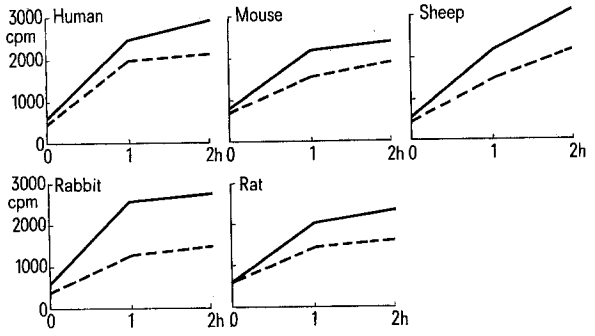

Fig. 3. Effects of pronase on $^{35}SO_4$ permeability on animal erythrocytes. ——, Control; ----, pronase 2 mg/ml.

The proteolytic enzymes are useful probes to determine which proteins of the membrane are exposed to the outside and potentially to determine the functional role of those proteins. The steric configuration of mouse glycoprotein may be similar to that of human glycoprotein. The glycoproteins of rat, sheep and rabbit erythrocytes are protective to these proteolytic enzymes. In human red blood cells, pronase reduces anion permeability, increases cation permeability and has no effect on the non-faccilitated component of the flux of the non-electrolyte. The protein is released much faster in the electrophoresis than the effects on anion permeability develope. There does not seem to be a causal relationship between the observed changes of the membranes' protein content and the permeability changes.

A mouse system for demonstrating the presence of inflammatory factors from human peripheral blood lymphocytes

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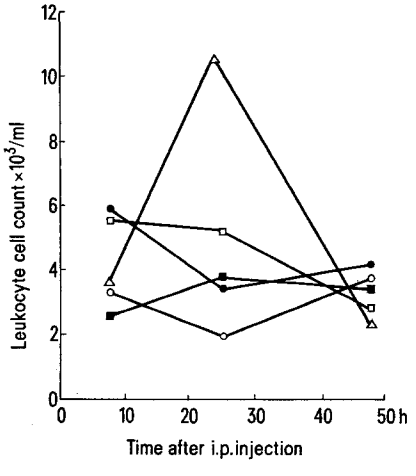
Summary. An assay system is described that allows the presence of inflammatory factors in supernatants from stimulated tuberculin-sensitive human peripheral blood lymphocytes to be demonstrated, by the induction of an inflammatory exudate in the peritoneal cavity of normal C57 BL mice.

The release of inflammation-inducing skin reactive factors (SRF) by antigen-stimulated sensitized animal and human lymphocytes has usually been demonstrated by the injection of active culture supernatants into the skin of normal unimmunized animals, e.g. guinea-pigs[2–6]. Although such a method allows the area of the inflammatory reaction induced to be assessed quantitatively, the type and number of cells infiltrating the test area can only be determined by microscopic examination of stained sections of the skin. We report here briefly a technique for the assay of inflammatory factors from human lymphocytes utilizing the ability of active supernatants to induce an exudative response in the peritoneal cavity of normal unimmunized mice.



Inflammatory exudates produced in mouse peritoneal cavities at various times after the injection i.p. of supernatants from sensitized lymphocyte cultures and control cultures. △—△, lymphocyte cultures incubated with PPD 0.5 μg/ml; ●—●, lymphocyte cultures incubated without antigen; ○—○, killed lymphocytes incubated with PPD 0.5 μg/ml; □—□, culture medium culture incubated with antigen; ■—■, culture medium culture incubated without antigen.

Materials and methods. Peripheral venous blood was obtained from healthy adults of either sex exhibiting delayed hypersensitivity skin reactions to tuberculin PPD. Peripheral blood lymphocytes were obtained by sedimenting the blood with a 6% solution of Dextran T110 (Pharmacia) in saline, 1 ml of dextran to 10 ml of blood. The leukocyte-rich supernatants were pipetted off and after washing were incubated at a cell concentration of 0.6×10^6 lymphocytes/ml in 2 ml of RPMI 1640 (GIBCO) containing penicillin 100 units/ml and streptomycin 100 μg/ml (GIBCO) with 20% heat inactivated autologous plasma. Cultures were stimulated with PPD (Connaught Laboratories) 0.5 μg/ml and control tubes either had an equal volume of saline added to the cells in culture instead of antigen, or PPD was added after the cells had been killed by heat at 60 °C for 30 min. Viability was checked by trypan blue exclusion. A further control consisted of culture medium alone or with antigen added but without cells. Supernatants were removed after 3 days incubation at 37 °C in sterile glass culture tubes in triplicate in 5% CO_2 and air. The supernatants were cleared of cells by centrifugation at 1000 × g for 10 min and were stored at −20 °C until used.

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Inflammatory exudate produced 24 h after i.p. injection in normal mice by supernatants from sensitized human lymphocyte cultures and by control supernatants

Culture supernatants		Stimulated cells	Unstimulated cells	Killed cells	Medium and antigen	Medium control
Cell Count $\times 10^3$/ml		7.12 ± 2.3	2.3 ± 0.9	2.3 ± 0.6	1.9 ± 0.9	3.4 ± 2
t-test			$p < 0.01$	$p < 0.01$	$p < 0.01$	< 0.05
Differential	Polymorphs	4	0	0	0	0
Count	Lymphocytes	74	88	91	85	95
Percent	Monocytes	22	12	9	15	5

Unconcentrated supernatants from the stimulated and control leukocyte cultures were injected i.p. in 1 ml aliquots into normal C57 BL mice anaesthetized with ether. There were 6 animals in each treatment group. The mice were sacrificed at 8, 24 and 48 h after the initial injection, each animal being injected i.p. with 1 ml of warm RPMI 1640 1 h prior to sacrifice. Subsequently, reactions were assessed at 24 h only. After sacrifice, any fluid contained within the peritoneal cavity was withdrawn using a blood diluting pipette, a drop of the fluid was smeared upon a slide for later fixation and staining and the remaining fluid was used to carry out a white cell count using a hemocytometer.

Results and discussion. The i.p. injection in mice of supernatants from the human lymphocyte cultures, as indicated in the figure, resulted in a peak in the inflammatory exudate produced that occurred at 24 h with supernatants from sensitized lymphocytes cultured with PPD. At other times and with control supernatants, the cellularity of the exudate remained approximately the same.

In further experiments, the nature of the exudate occurring 24 h after i.p. injection of the culture supernatants was examined. It was found that supernatants from sensitized human lymphocyte cultures stimulated with PPD produced exudates with significantly greater cellularity than did those from control supernatants (table). The differential counts obtained from fixed and stained smears of the exudates did not appear to differ markedly between groups. There were more monocytes and fewer lymphocytes, and some polymorphonuclear leukocytes present in the exudates developing in response to supernatants from stimulated cultures, as compared with the exudates induced by control supernatants. Supernatants from a number of donors with approximately equal tuberculin sensitivity were assessed using the assay system. All supernatants induced an inflammatory exudate of similar cellularity, in that cell counts of exudates induced by stimulated lymphocyte culture supernatants were approximately 3 times those induced by unstimulated control supernatants. A stimulated culture supernatant concentrated seven times by lyophilization produced an exudate with 7 times the cellularity of that induced by the unconcentrated supernatant (42×10^3 cells/ml compared to 5.92×10^3 cells/ml).

There thus appeared to be evidence for the presence of inflammatory factors in PPD stimulated lymphocyte culture supernatants. These supernatants produced an exudate peaking at 24 h. In addition, there was a tendency for such supernatants to induce an inflammatory response containing more monocytes and polymorphs than that seen with contol supernatants. This would not be unexpected if the active supernatants contain factors such as the macrophage migration inhibition factor (MIF)[7] and the polymophonuclear leukocyte inhibitory factor (LIF)[8] that have been demonstrated by in vitro techniques. It should be stressed that the ability of these supernatants to stimulate an inflammatory exudate may or may not reflect SRF activity. Nevertheless, the data presented do demonstrate that soluble factors from human lymphocytes will induce an inflammatory response in the mouse that may be characterized and quantitated by this assay system.

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Acceleration of methemoglobin reduction in erythrocytes by selenium

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Summary. Selenium accelerated the reduction of methemoglobin in erythrocytes. Its mode of action is suggested as a catalysis of the methemoglobin reduction by glutathione.

It was reported that dietary selenium prevents not only hemolysis, but also oxidation of hemoglobin to methemoglobin induced by hydrogen peroxide or ascorbic acid[3–5]. Since it was recently demonstrated that selenium forms an integral part of the glutathione peroxidase molecule[5,6] whose activity is proportional to the amount of dietary selenium[7], the prevention of oxidative damage of erythrocytes by selenium has been suggested to be associated with this enzyme activity in the cells. This paper reports the role of selenium in preventing oxidative damage of hemoglobin, showing that selenium accelerates the reduction of methemoglobin in intact rat erythrocytes.

Materials and methods. Male Sprague-Dawley rats were decapitated and their blood was collected in heparinized tubes and centrifuged at $500 \times g$ for 10 min at 4 °C. The plasma and buffy coat were removed and the erythrocytes were washed 3 times with isotonic phosphate buf-