affinity receptors are connected with the process of lymphocyte activation. If, as suggested, the high-affinity receptors consist of two low-affinity receptors, it means that lymphocytes, in order to become activated, require the bivalent attachment of the stimulating molecule to the cell membrane. Leucoagglutinin in higher concentrations react predominantly with the low-affinity receptors or monovalently and does not stimulate lymphocytes as shown previously[2].

It has been shown that leucoagglutinin does not have to be internalized in the lymphocytes during stimulation[3]. Thus the stimulatory process is initiated by the establishment of a dynamic equilibrium between lymphocyte receptors of high-affinity type and the mitogen molecules.

Zusammenfassung. Es wurde die Kinetik der Reaktion zwischen Lymphozyten und Leukoagglutinin aus *Phaseolus vulgaris* untersucht.

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Thermal Injury: Release of a Cytotoxic Factor

Recent studies from these laboratories have suggested the presence of a specific inhibitor of muscular contraction in the serum of acute thermally injured patients[1]. A toxic glycoprotein isolated from in vitro scalded human skin inhibited the formation of adenosine 5'-triphosphate (ATP)-induced tension by glycerol extracted muscle fibers[2,3]. Earlier studies have shown that the 'toxic glycoprotein', when administered i.v., proved lethal to mice, and, when incorporated into the growth medium, inhibited the growth of Hela and HEP_2 cells[4].

Sensitized lymphocytes are known to mediate allograft and tumor immunity as well as delayed hypersensitivity reactions. Lymphocytes-mediated-cytotoxicity against various target cells can be induced by several methods which include the addition of lymphocytes specifically sensitized against the corresponding target cells, or the addition of antigen unrelated to the target cells, to a mixture of target lymphocytes specifically sensitized against the added antigen. Investigation of the mechanisms of cell-mediated immune reactions and induction of lymphocyte-mediated cytotoxicity against various target cells in various types of trauma, viral and neoplastic is valuable for the understanding of the cell-mediated reactions[5–8] and of the immunological state of the victim.

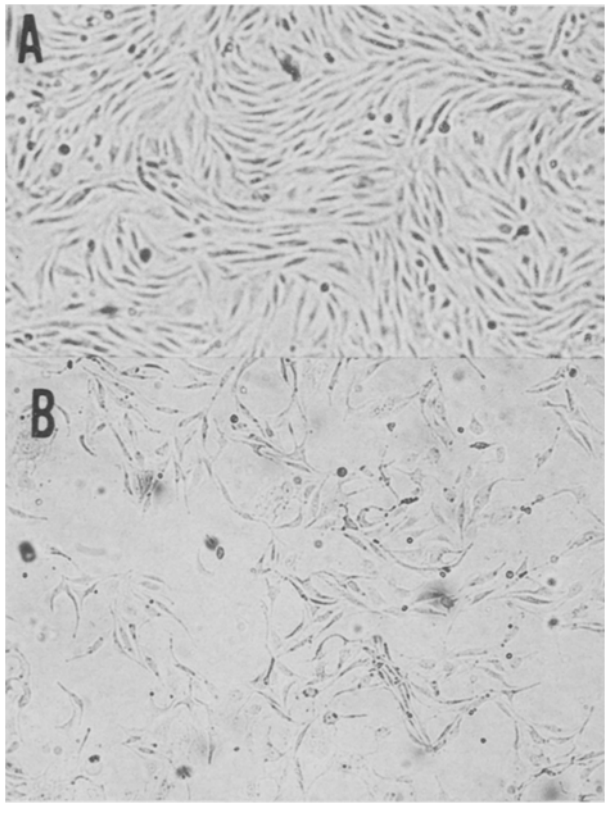

Fig. 1. Cytotoxic effect of the 'acute burn serum inhibitor' on rabbit heart fibroblasts: 48 h growth of rabbit heart fibroblasts in basal Eagles medium in presence of 0.10 ml of extract from normal serum (A), and from serum of thermally injured patient with 60% total body injury.

The present study was performed to investigate the cytotoxic effects of an 'acute burn serum inhibitor' isolated from the serum of acute thermally injured patients, on fibroblast of rabbit heart culture, and on the growth and migration of lymphocytes from normal and thermally injured subjects.

Methods. Blood samples were obtained from Sumner L. Koch Burn Ward at Cook Country Hospital. The blood was allowed to clot at room temperature, and the serum was separated at $1000 \times g$ for 15 min. The serum (10 ml) was diluted with an equal volume of physiological saline, then mixed with one-fifth volume of glacial acetic acid and an equal volume of butanol. The aqueous phase was separated after centrifugation for 60 min at $1000 \times g$, and processed as described for the 'toxic glycoprotein'[4,9]. The 'acute burn serum inhibitor' was finally dialyzed against physiological saline and made up to a final volume of 10 ml.

Fibroblasts of primary heart cultures were obtained from albino rabbits, 3–4 lbs. The hearts were removed, rinsed free of blood and cut into small pieces in 10 ml of Earle's salt solution. The solution then decanted, and the

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tissue was placed in 20 ml of 0.5% trypsin in Earle's salt solution. The tissue was shaken for 30 min, then centrifuged at $1000 \times g$ for 6 min. The supernatant was discarded and the cells were resuspended in 5 ml of fresh medium in presence and absence of the 'acute burn serum inhibitor'.

Human lymphocytes were obtained from the buffy layer of freshly drawn heparinized human blood, were washed, filled in heparinized capillary tubes and placed on the bottom coverslip of a Mackanese type chamber[10] and fixed with parafilm. The lymphocytes from normal blood were incubated in Eagle's minimum essential medium (MEM) without, and with either 0.05 ml (1.0 mg/ml) of the 'toxic glycoprotein' from in vitro scalded human skin, or 0.05 ml of normal serum or of the 'acute burn serum inhibitor' at 37 °C for 24 h. Duplicate capillaries were used for each test. The area of cell migration was calculated as the product of 2 diameters of the migration zone. The percentage of inhibition of migration was calculated as equal to:

$$100 = \frac{\text{Area of migration in experimental chamber}}{\text{Area of migration in control chamber}} \times 100$$

Results. Solutions of the 'acute burn serum inhibitor' in 0.9% NaCl did not show any absorption peak when examined in the ultraviolet range with the use of a Beckman Model DU2 spectrophotometer. The lack of an absorption peak between the wavelength of 3,000 and 2,650 Åuggested the absence of purines, pyrimidines or nucleic acid. Also the lack of an absorbtion peak between the wavelengths of 2,750 to 2,880 Å indicated the absence of tryptophane or tyrosine. Digestion with trypsin followed by resolution on Whatman No 1 filter paper, the 'burn serum inhibitor' produced ninhydrin reacting compounds, and if incubated with collagenase it reacted with anthrone sulfuric acid, and gave the characteristic orangered color when interacted with orcinol, suggesting the presence of carbohydrates. The sedimentation pattern of the 'acute burn serum inhibitor' in 0.9% NaCl, showed 1 single peak, sedimenting with a velocity of $S_{w,20}$ of 3.05.

Figure 1 suggests that, when incorporated into the growth medium, the 'acute burn serum inhibitor' depressed the growth of rabbit heart fibroblasts.

The data summarized in Table IA suggests that the migration of lymphocytes cells from acute thermally injured patients was markedly slower than that of the lymphocyte from normal volunteers. Figure 2 A shows typical migration of lymphocytes from thermally injured patients compared with that of normal cells.

The migration of normal lymphocytes was markedly inhibited in the presence of 0.05 mg of the toxicglyco-

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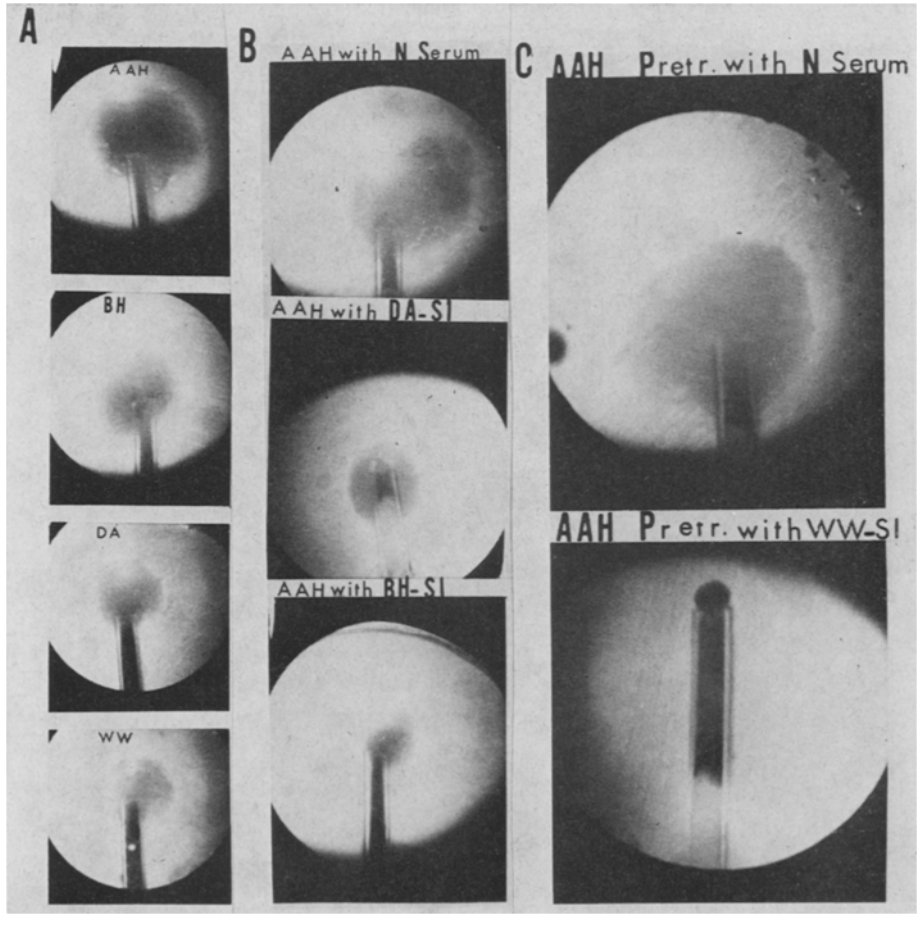

Fig. 2. A) Migration of lymphocytes from normal blood (AAH), and from blood of acute thermally injured patients BH, DA and WW. B) Migration of cells from normal blood (AAH) allowed to grow in presence of normal serum, and of the 'acute burn serum inhibitor' from patients DA and BH. C) Migration of normal lymphocytes pretreated with normal serum (upper), and with the 'acute burn serum inhibitor' (lower) for 10 min, washed, placed into capillaries. then allowed to migrate in fresh Eagle's minimum essential medium.

protein', and in the presence of the 'acute burn serum inhibitor' as shown in Figure 2B. Figure 2C suggest that, when pretreated with the 'acute burn serum inhibitor', then washed and allowed to migrate in fresh MEM, normal lymphocytes failed to migrate. The results of these experiments, summarized in Table IB, suggest that the 'acute burn serum inhibitor' reacts with the lymphocytes. The magnitude of the migration inhibition varied from one patient to another.

Discussion. When incorporated into the growth medium, the 'acute burn serum inhibitor' depressed the in vitro growth of rabbit heart fibroblasts. If added to the Earle minimal essential medium, it inhibited the migration and growth of the normal lymphocytes. Lymphocytes of acute thermally injured patients migrated at slower rate than the normal lymphocytes. The speed of migration of these lymphocytes varied from one injured patient to another, and possibly depended on the magnitude of the thermal injury. These observations indicate the release of cytotoxic antigen into the blood of the injured patients, and suggest an impairement of cellular immunity in the acute thermally injured patients, which is in agreement with the findings from other laboratories[11,12]. MESSERSCHMIDT[11] showed disorders of antibody formation in mice following burns and whole body irradiation, and QUISOMORIO[12] described the occurence of rheumatoid factors, antinuclear antibodies and antiepithelial antibodies in thermal injury.

It is obvious that a toxic antigen released into the circulation as a result of thermal or other type of insult, i.e. trauma, viral or neoplastic, will bind at the surface or on the plasma membrane of the lymphocytes leading to cytotoxic reactions in immunologically specific and non-specific cells. The evidence cited above suggests that the 'acute burn serum inhibitor' acted directly on the lymphocytes. This interaction could lead to the blocking of receptors on the lymphocytes which are necessary for recognition of foreign antigens, and the induction of a non-responsive, depressed state in the reticuloendothelial system of the victims[13,14].

A) Migration of human lymphocytes from acute thermally injured patients

Lymphocytes from	Control migration (%)
Normal	100
Thermally injured patients	
D.A.	62
B.H.	39
W.W.	70
T.R.	10
K.L.	40

B) Migration inhibition of normal human lymphocytes

Factor in medium	Inhibition (%)
Normal saline	0 (10)[a]
Normal serum	0 (15)
Toxic glycoprotein (50 µg)[b]	98 (14)
Toxic glycoprotein (25 µg)	85 (9)
Toxic glycoprotein (5 µg)	75 (10)
Acute burn serum inhibitor (5 µg)	
D.A.	77 (5)
W.W.	95 (8)
B.H.	85 (10)
Acute burn serum (0,05 ml)	
D.A.	85 (6)
W.W.	85 (6)
T.R.	76 (10)
K.L.	60 (8)

[a] Number in paranthesis are munber of aliquots assayed. [b] Amount used per ml of medium. All the sera were used after inactivation at 56 °C for 30 min.

Résumé. Un facteur cytotoxique fut obtenu du serum des malades qui ont été fortement brûlés. Il a produit l'inhibition et l'agglutination des fibroblastes cardiaques du lapin et a inhibé la migration des lymphocytes humains.

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The Fate and Effects of *Salmonella* Flagellin in Neonatal Rat Intestine

The biological distribution and fate of antigens injected s.c. or i.v. has been extensively studied[1-3]. In contrast, there is very little information about the fate or effect of antigens which occur naturally, particularly in the neonatal period. Studies of antigens entering through the surfaces of the intestine and lungs have been limited to immunologically mature animals[4-7] and studies of antigen in neonatal animals have been limited to injected material[8,9]. Antigenic proteins as large as ferritin are absorbed through the intestinal mucosa of neonatal animals[10,11], and this absorption has selectivity and is age-related[12]. We have started to study the immunological effect and the biological fate of intestinally absorbed macromolecules in neonates.

Materials and methods. Spraque-Dawley rats aged 1–20 days were used. The mothers received in drinking water potassium iodine, 1 mg/l, for 7 days prior to parturition and until the neonates were killed. Monomeric flagellin was prepared from *Salmonella* Derby, American Type Culture Collection, Maryland, and labelled with carrier free I^{125} by the method of ADA et al.[13]. 25 µg labelled flagellin in 0.1 ml of a diluted suspension of colloidal carbon (marker substance) were delivered into the distal half of the stomach through a polyethylene tube. Rats studied were 1, 5, 10, 15 and 20 days of age. Rats were killed at times varying from 3 to 120 h after antigen administration. There were 6 to 10 rats killed at each time interval. The intestine with attached mesentery and lymph nodes, spleen, thymus, liver and lungs were rinsed in saline three times to remove free iodide, counted using a scintillation crystal and Baird-Atomic spectrometer, fixed in 10% formalin and then prepared as microscopic sections for autoradiography with Kodak NTB 3 emulsion. Comparisons were made with the distribution