The Uptake of Iodinated Protein by Spleen Cells of Guinea-Pigs in vitro[1]

Relatively little information is available about the amount and the kinetics of the uptake of proteins by mammalian cells in vitro[2-5]. To obtain data in this field on the quantitative relationship between uptake of soluble antigen and induction of antibody synthesis, we studied the uptake of bovine serum albumin (BSA) and bovine serum γ-globulin (BGG) by normal, non-immunized spleen cells of guinea-pigs in vitro. The BSA and BGG was labelled with J 125[6].

Material and methods. Guinea-pigs, 200–250 g, both sexes, from different dealers, were fed with dry food and sacrificed by heart puncture for the preparation of spleen cells. After washing the isolated spleen cells in saline, 3×10^7 spleen cells were suspended in 4.5 ml Hanks balanced salt solution with J 125-BSA 6×10^{-10} to 1.0×10^{-4} g or J 125-BGG 1.0×10^{-7} to 1.0×10^{-6} g in 0.5 ml Hanks balanced solution at 37 °C or 4 °C. Incubation time was 10 sec to 60 min. After incubation the cells were washed 4–8 times in saline and resuspended in 0.5 ml saline. The J 125 activity of the cells and of the different supernatants after the washing were counted in triplicates in LB 200 Beckman in dioxan-naphthalin-PPO. Labelling of BSA and BGG was done by Dr. HAMMER, Max-Planck-Institut für Immunbiologie Freiburg: 1.242 mg BSA + 5 mC J 125 = 1 γ BSA gave 185,500 cpm; 1.067 mg BGG + 3 mC J 125 = 1 γ BGG with 208,060 cpm. The vitality test for the spleen cells before and after incubation was done with trypane blue: 0.1 ml cell suspension + 0.1 ml 1% solution trypane blue + 0.8 ml saline; the blue stained cells were counted in a Thomas counting chamber.

Results. A great number of experiments was performed. The following are a few of the typical results:

(1) In a basic experiment 3×10^7 spleen cells were incubated for 60 min at 37 °C with 10^{-10} g J 125-BSA. The amount taken up by the spleen cells was 10^{-12} g J 125-BSA or 1%. From 10^{-7} g J 125-BGG 3×10^{-7} spleen cells took up about 10% or 6×10^{-8} g. This difference in the amount of BSA and BGG taken up by spleen cells could be explained by the different sizes of the molecules of the former[7].

(2) The 1% relation between offered and incorporated J 125-BSA remains constant if the amount of BSA is increased to 10^{-4} g J 125-BSA for 3×10^7 spleen cells. If the spleen cells are incubated with 12 g% unlabelled BSA and then incubated with 10^{-7} g J 125-BSA, again 1% of the J 125-BSA is taken up by the spleen cells[2] (Figure 1). This 1% relation is also not altered if the spleen cells are incubated in normal guinea-pig serum.

(3) The uptake of J 125-BSA and J 125-BGG by spleen cells decreases after an incubation of 60 min at 4 °C: from 10^{-5} g J 125-BSA at 37 °C incubation temperature 1.0×10^{-7} g is taken up by 3×10^{-7} spleen cells, at 4 °C only 0.67×10^{-7} g J 125-BSA is taken up. The inhibition of the uptake is 33%. For BGG the inhibition is 40%: the spleen cells have taken up only 6×10^{-9} g J 125-BGG instead of 1.0×10^{-8} g from 1.067×10^{-7} g BGG. This decrease is probably due to the inhibition of some process in the uptake of soluble protein by cells which requires energy. At 23 °C incubation the uptake of BSA or BGG is not inhibited.

(4) In a further experiment we tested the importance of the amount of uptake in intact spleen cells. 4×10^7 spleen cells were kept at 37 °C in saline for 1–24 h. After counting the trypane blue positive cells, the different samples of spleen cells were incubated for 60 min at 37 °C in 1.0×10^{-6} g J 125-BSA.

If all cells were trypane blue positive 0.36% of the amount offered was taken up, but if 75% of the cells were trypane blue positive 0.88% was taken up; at 30 and 20% it was 0.75%. In our 'normal' experiment 10–20% of the cells were trypane blue positive after the incubation with J 125-BSA or J 125-BGG. These results indicate that a great number of trypane blue positive cells do not increase the uptake of BSA or BGG. On the contrary, with 100% trypane blue positive cells the uptake was inhibited[8].

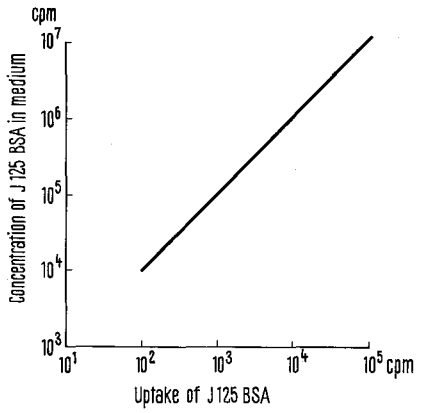

Fig. 1. Linear increase of uptake of J 125-BSA with increasing concentration in the incubation solution. 1.0×10^6 cpm = 2×10^{-6} g J 125-BSA.

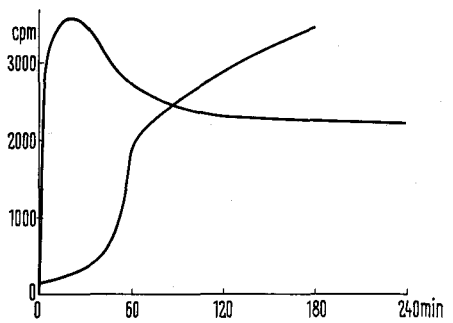

Fig. 2. Kinetics of J 125-BSA: within seconds, the uptake begins and finishes in about 2 min. Excess of uptake after 10–20 min (for comparison, the slow increasing curve at the right is the uptake of brucella antigen by normal spleen cells). 1.0×10^6 cpm = 2×10^{-6} g J 125-BSA.

[1] The work was supported by a grant of the Stiftung Volkswagenwerk and of the Deutsche Forschungsgemeinschaft.

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(5) The kinetics of the uptake of BSA and BGG was studied by a modification of the incubation technique in order to measure very short incubation times from 10 sec up to 60 min. 3×10^7 spleen cells were incubated at 37 °C with 1.0×10^{-6} g BSA. After 10 sec 2500 cpm were counted on the washed spleen cells or 0.5×10^{-8} g BSA. After 30 sec 3500 cpm and after 120 sec 4700 cpm were counted. This means that in 2 min nearly the whole amount of BSA is taken up by the cells, since the amount after 30 min incubation time is 4750 cpm. This amount remains constant throughout 2 h incubation. This result indicates that the BSA molecule is absorbed in seconds on the cell surface (Figure 2).

All our experiments showed after 10–20 min incubation an excess of uptake which disappeared a few minutes later. This excess of uptake is not observed if the cells are incubated with brucella antigen (Figure 2).

Zusammenfassung. 6×10^{-10} g bis 10^{-4} g J 125 Rinderserum-Albumin (BSA) bzw. $1{,}0 \times 10^{-7}$ bis $1{,}0 \times 10^{-6}$ g J 125 Rinderserum-γ-Globulin (BGG) werden 3×10^7 Milzzellen normaler Meerschweinchen in vitro angeboten. Unabhängig von der Höhe des Angebotes beträgt die Menge des aufgenommenen BSA 1%, die des aufgenommenen BGG 10% des Angebotes. Durch 4 °C Inkubationstemperatur wird die Aufnahme z.T. behindert. Die Aufnahme findet nach wenigen Sekunden Inkubationszeit statt und ist nach etwa 2–3 min abgeschlossen.

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Distribution of Procoagulant Activity in the Subcellular Fractions of Human Granulocytes

Procoagulant activities in the leucocytes were found by several authors[1,2]. Lisiewicz[2] stated that this activity has similar features to that of platelet factor 3. The granulocytes possess also an anticoagulant activity[3,4]. Saba et al.[4] demonstrated that this activity is connected with lysosomal cationic proteins. The purpose of this work was to study the distribution of procoagulant activity in the subcellular fractions of human granulocytes.

Material and method. The granulocytes were obtained from human purulent pleural exudate. The Cohn and Hirsch method[5] was used to obtain the following subcellular granulocyte fractions: I, sediment after 400 *g*; II, sediment after 8200 *g*; III, supernatant after 8200 *g*. Fraction I contains nuclei, membranes and cell debris, large specific granules; fraction II, specific granules (lysosomes); fraction III, smaller cytoplasmic alements: small specific granules, mitochondria, ribosomes, endoplasmatic reticulum and Golgi apparatus fragments.

All granulocyte fractions and granulocyte homogenates were suspended in a solution of 0.34 *M* saccharose. They were frozen and thawed 6 times, homogenized 1–2 min using a Potter homogenizer. All the preparations were dialysed against a phosphate buffer (0.025 *M*; pH 7.65; +4 °C; 18 h). Human plasmas deficient in factor VIII, IX and XII were obtained from patients with hereditary deficiencies of these factors. Factor VIII, IX and XII were determined according to the method of Soulier and Larrieu[6], slightly modified by Niewiarowski et al.[7].

In order to compare the procoagulant activity of human plasma with that of the granulocyte suspension, the granulocyte suspension was diluted to a concentration of 6000 cells/μl. The procoagulant activity was tested simultaneously in the granulocyte suspension and in the plasma and the dilution curves were made. Each clotting factor activity in the granulocyte suspension was expressed as % of the activity of the same factor, found in the equal volume of plasma (accepted as 100%). Furthermore, the specific activity of each clotting factor was calculated according to the formula: $1000/(t \times p)$ where t = clotting time in sec, p = protein concentration (mg/ml).

Result and discussion. The results are summarized in the Table. It can be seen that the relative procoagulant activity of the homogenate and of subcellular fractions is of the same order of magnitude as that of normal plasma. The specific procoagulant activity of the homogenate is considerably higher than of plasma. It is higher in all isolated subcellular fractions, particularly in fraction II and III, than in the whole homogenate. This fact may be explained by the presence of anticoagulant activity which is diminished by dilution during fractionation. Factor XII-like activity can be adsorbed partially

Distribution of procoagulant activity in subcellular fraction of human granulocyte[a]

Granulocyte fraction		Substrate plasma						
		Factor VIII deficient		Factor IX deficient		Factor XII deficient		
		A	B	A	B	A	B	C
Total homogenate		116%	11.8	51%	8.1	43%	7.1	–
Sub-fractions	I	112%	20.4	40%	12.6	46%	11.7	–
	II	77%	43.7	41%	36.9	49%	36.4	17.8
	III	190%	147.2	26%	56.6	38%	59.0	17.0
Human plasma		100%	1.8	100%	2.4	100%	1.7	–

[a] Mean values from 5 determinations. A, relative activity as compared with normal plasma; B, specific activity $1000/(t \times p)$; C, specific factor XII-like activity after adsorption of tested material on kaolin and elution at alkaline pH.

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