Zusammenfassung. Die Anzahl der Teilungsprozesse transduzierter Zellen im Vergleich zu nichttransduzierten Zellen nimmt während der ersten fünf bis sechs Zellteilungen in der Kultur um zwei bis vier Teilungen ab. Dieser Unterschied wird offensichtlich durch das regelmässige Abstossen von Zellen vom gleichen Typ wie beim Akzeptorstamm von potentiell transduzierten Zellen bewirkt. Die grundlegenden Mechanismen des Transduktionsprozesses werden diskutiert.

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On the Fate of Rabbit Antibody/Antigen Complexes in the Presence of Normal Leucocytes *in vitro*[1]

Several investigators have shown by experiments *in vivo* that antigen is eliminated more rapidly in immune than in normal animals (for references see[2]), a fact presumably dependent upon the formation of variously composed antibody/antigen complexes. It has also been shown that passively transferred antibody is degraded faster when the injection of antibody is preceeded by an injection of antigen[3]. Both HUMPHREY[4] and WEIGLE[5] have also recently reported that when both components of antibody/antigen complexes are injected intravenously to normal rabbits, they are rapidly eliminated.

It has been demonstrated in earlier communications that rabbit antibody/antigen complexes are taken up and broken down by normal guinea pig leucocytes *in vitro*[2,6] and that spleen cells from immune animals adsorb considerably more antigen than cells from normal animals[7]. This latter fact is presumably mainly due to antibody fixed on cell surfaces.

The present note reports on the *in vitro* fate of trace-labelled rabbit antibody/antigen complexes in the presence of leucocytes from peritoneal exudates of normal guinea pigs and rabbits. The purpose of the investigation was to establish (1) whether both antigen and antibody are taken up and broken down, and (2) whether the fate of antibody in homologous cells was different to the fate in heterologous cells. Human serum albumin (HSA) was used as antigen. Two types of insoluble complexes were prepared: (a) A complex comprising both carrier and trace-labelled I^{131}-HSA and unlabelled rabbit antibody. (b) A complex comprising unlabelled HSA and internally labelled rabbit C^{14}-antibody. The C^{14}-antibody was obtained by incubating spleen fragments from hyperimmune rabbits (boosted 4 days previously with the antigen, HSA) with C^{14}-amino acids in Gey's balanced salt solution *in vitro*[7,8]. Rabbit C^{14}-antibody was isolated by precipitation with unlabelled HSA in the presence of carrier antibody. Both types of insoluble complexes contained similar amounts of antibody and antigen in the region of slight antigen excess.

Exudate cells were taken 3 days after intraperitoneal injection of glycogen in saline. For the study of uptake and breakdown identical amounts of the labelled antibody/antigen complexes (100 μg protein) were incubated with 0.5 ml of a 10% suspension of either guinea pig or rabbit cells in Gey's solution containing 10% homologous serum (Medium) for 2 h at 37°C. Controls contained Medium without cells. To exclude the possibility that the degradation of the complexes occurred extracellularly, controls were set up with supernatants from cell suspensions preincubated for 2 h at 37°C. The cells were centrifuged down and the supernatants incubated with the antibody/antigen complexes.

To terminate the experiments, the mixtures were centrifuged and 0.2 ml of a 25% trichloracetic acid solution was added to the clear supernatants. After 15 min the mixtures were centrifuged and the radioactivity of the supernatants determined. The results of a typical experiment are shown in the Table.

The controls show that incubation of cells for 2 h did not release sufficient proteolytic enzyme to degrade the antibody/antigen complexes extracellularly. The breakdown which occurred in the presence of the cells is therefore assumed to take place intracellularly following phagocytosis of the complex.

Catabolism of antibody/antigen complexes by leucocytes *in vitro*

Type of cells	Type of antigen/antibody complex	Breakdown of antigen or of antibody(%)[a]
Rabbit leucocytes	Rabbit antibody/I^{131}-HSA	13
	Rabbit C^{14}-antibody/HSA	9
Supernatant control after cell removal	Rabbit antibody/I^{131}-HSA	0
	Rabbit C^{14}-antibody/HSA	0
Guinea pig leucocytes	Rabbit antibody/I^{131}-HSA	6
	Rabbit C^{14}-antibody/HSA	5
Supernatant control after cell removal	Rabbit antibody/I^{131}-HSA	0
	Rabbit C^{14}-antibody/HSA	0
Gey's 10% serum control		0

[a] These values were obtained by calculation from the radioactivity of the supernatants after precipitation by TCA.

[1] Studies on the Fate of Antigen *in vitro*. II.

[2] E. SORKIN and S. V. BOYDEN, J. Immunol. *82*, 332 (1959).
[3] S. P. MASOUREDIS, L. R. MELCHER, and M. B. SHIMKIN, J. Immunol. *71*, 268 (1953).
[4] J. H. HUMPHREY, in *Mechanisms of Antibody Formation*. Proceedings of a Symposium (Publishing House of the Czechoslovak Academy of Sciences, Prague 1960), p. 39.
[5] W.O.WEIGLE, in *Mechanisms of Antibody Formation*. Proceedings of a Symposium (Publishing House of the Czechoslovak Academy of Sciences, Prague 1960), p. 53.
[6] C. G. COCHRANE, W.O.WEIGLE, and F. J. DIXON, J. exp. Med. *110*, 481 (1959).
[7] E. SORKIN, J. M. RHODES, and S. V. BOYDEN, J. Immunol. *86*, 101 (1961).
[8] B. A. ASKONAS and J. H. HUMPHREY, Biochem. J. *68*, 252 (1958).

It is apparent that antibody as well as antigen is broken down by the cells. This enzymatic degradation of rabbit antibody occurs both in rabbit and guinea pig cells.

Zusammenfassung. Exsudatzellen von normalen Meerschweinchen und Kaninchen phagocytieren und spalten *in vitro* unlösliche, radioaktiv markierte Antikörper/Antigen-Komplexe. Die Kaninchenantikörper werden sowohl durch Kaninchen- wie auch Meerschweinchenzellen enzymatisch abgebaut.

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Evidence that No Significant Quantities of *p*-Amino-Hyppuric Acid are Transported by the Renal Lymphatics

One of the basic assumptions in the measurement of renal plasma flow by the *p*-amino-hyppuric acid (PAH) clearance is that all PAH removed from the blood during its transit through the kidney is excreted in the urine. The equation for the calculation of renal plasma flow (RPF) would however be incorrect if substancial amounts of PAH were removed by the renal lymphatics. The proper equation would then be

$$RPF = \frac{UV - LF}{A - R}$$

where U, L, R and A are concentrations in the urine, renal lymph, renal venous and arterial plasma; V urine volume and F lymph flow. Assuming that L is low compared to U (approximately equal to R) and F small compared to RPF, LF can be neglected.

BÁLINT *et al.*[1] have, however, demonstrated that values for renal blood flow as calculated from the PAH clearance and extraction ratio are often markedly lower than those established by measuring renal venous outflow. Very large differences were observed especially at low urine flows as produced by haemorrhagic hypotension.

The discrepancies may be due to tubular conjugation, or accumulation of PAH in the renal parenchyme. The possibility of lymphatic loss of PAH must however be raised.

If a significant amount of PAH is transported by the renal lymphatics, it must consequently raise the concentration of the substance in the thoracic duct lymph well above plasma levels. We therefore measured in dogs, during an intravenous infusion, the respective concentrations of PAH in blood plasma and thoracic duct lymph. The renal clearence of the *p*-amino-hyppuric acid was determined at the same time.

Even after intralymphatic infusions in dogs with thoracic duct fistula, a certain amount of PAH appears in the urine[2]. It can be assumed that some of the infused PAH on its way from the renal lymphatics to the thoracic duct diffuses through the wall of lymphatic vessels and is subsequently absorbed by the capillaries.

It therefore seemed necessary to measure, in some experiments, the concentration of PAH in renal lymph simultaneously with plasma and thoracic duct lymph concentrations.

Our experiments confirmed that the concentration of PAH in thoracic duct lymph never exceeds, by a significant difference, the concentration in blood plasma. The 'lymphatic clearence' as calculated by the equation

$$C^L_{PAH} = \frac{L \cdot F_t}{A}$$

of PAH is negligible as compared with the renal clearence (Table). Haemorrhagic hypotension and consequent low urine flow does not increase the lymphatic clearence. The concentration of PAH in renal lymph is not higher than in thoracic duct lymph or blood plasma and it does not

A	L		U	C_{PAH}	C^L_{PAH}	
	Thoracic duct	Renal				
0.70	0.84		140		0.36	
1.53	1.83		218	36	0.56	
2.09	3.60		231	38	1.16	
2.29	3.83		278	32	1.67	Hypotension
2.09			280	4		Hypotension
0.84	0.67		218	70	0.12	
0.80	0.86		218	98	0.14	
0.85	0.70		272	22	0.13	Hypotension
0.85	0.81		696	19	0.40	Hypotension
0.89	0.72		231	182	0.45	
0.85	0.90		198	233	0.48	
0.85	0.73		134	262	0.43	
0.77	0.98		220	63	1.59	Hypotension
0.75	0.93		283	208	1.30	
0.50	0.43		278	155	0.11	
0.54	0.44		220	138	0.19	
0.65	0.44		174	109	0.16	
0.70	0.51		382	20	0.31	Hypotension
0.30	0.77		405	11	1.03	Hypotension
0.62	0.67		280	171	1.50	
0.84	0,67		535	125	0.85	
1.24	1.13		590	170	2.22	
1.43	1.22		633	133	0.13	Hypotension
1.22	1.17		655	36	0.10	
0.75	1.10	0.90			1.36	
2.15	1.56				0.73	
0.61	0.75				0.38	
0.52	0.58	0.77			0.12	
0.46	0.49	0.50			0.10	
0.45	0.44	0.39			0.29	Hypotension
0.45	0.46	0.47			0.29	Hypotension
1.04	1.07	1.01			0.58	
1.25	1.07	0.87			0.45	
1.25	1.16	0.82			0.51	

[1] P. BÁLINT, Á. FEKETE, J. STURCZ, and Z. SZALAY, Kisérl. Orvostud. *12*, 20 (1960).

[2] I. RUSZNYÁK, M. FÖLDI, and G. SZABÓ, *Lymphatics and Lymph Circulation* (Pergamon Press, Oxford, London, New York, Paris 1960).