and Storbeck [2] assumed that the complex ions in concentrated copper chloride solutions were $CuCl_3^{2-}$. Later on Szabo [3] and Tschaltykjan [4] showed that in a solution where the mole ratio CuCl:MeCl is <0.6 the dominating ions are $CuCl_3^{2-}$. It may now be assumed that these ions react with acetylene to give for example $Cu_2ClC{\equiv}CH$ which adds chlorine, or in a more neutral medium behaves as an acid to give the ion $Cu_2ClC{=}C^-$, which would be the precursor for the formation of tetrachloro ethylene.

The reactions may be summed up in the following formulas:

$$HC{\equiv}CH + 4\ CuCl_2 \rightarrow ClHC{=}CCl_2 + 4\ CuCl + HCl$$

$$HC{\equiv}CH + 6\ CuCl_2 \rightarrow Cl_2C{=}CCl_2 + 6\ CuCl + 2\ HCl$$

This new reaction may be used for the industrial production of tri- and tetrachloro ethylene.[5] Most methods known to-day are using two reaction steps. They also involve great losses of chlorine. An advantage of this new reaction is that cheap hydrogen chloride + oxygen or chlorine may be used for the re-oxidation of cuprous chloride. It is also to be assumed that this new reaction will be suitable for the production of bromoethylenes, or mixed bromo-chloro-ethylenes.

Experimental. A hot (+ 97°C) solution of $CuCl_2$ (3 moles/liter) and LiCl (6 moles/liter) was continuously pumped in from the bottom of a column (height 100 cm, diameter 5 cm). About 25 % of the Cu^{2+} was pre-reduced to Cu^+. The pH was about 1.5 (this value was measured with a standard glass electrode on a sample which was diluted with an equal part of distilled water in order to avoid crystallization of CuCl). Acetylene (10 l/h) was pumped in from the bottom and the used chloride solution was withdrawn at the top of the column. There was also a trap for condensing the organic chloro compounds produced. The conversion of acetylene in this experiment was 90 % and the yield of trichloroethylene 80 %. Byproducts were tetrachloroethylene and dichloroethylenes.

Further information about this reaction will be published elsewhere.

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Specific Interaction of Adenine Nucleotides with the Amino Acid Transport Mechanism in the Nuclear Membrane

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The transport of amino acids into isolated calf thymus nuclei has been shown to require the presence of sodium ions. The process is specific for the L-form of the amino acids and has well-defined temperature and pH optima. Amino acids can be divided into groups whose members exhibit mutual competition in transport.[1] Low concentrations of adenine nucleotides in the medium reduce the transport by about 40 %.[2] The phenomenon has been further characterized and the inhibition found to be a specific property of adenine nucleotides. The transport mechanisms for L-alanine and L-arginine are inhibited to the same degree, whereas the diffusion of the amino acids remains unaffected.

Transport was measured by incubating the isolated nuclei with the ^{14}C-labelled compound and measuring the radioactivity in the acid-extract of the nuclei after washing them free of external isotope. The incubation time used was 10 min. The time for equilibration of the labelled compounds was over 20 minutes in all cases, so the values measured represent transport rates.

When nuclei were incubated with different nucleoside triphosphates, ATP reduced the transport of L-alanine by 38 % (Table 1, Expt. 1). Of the corresponding monophosphates only AMP was found to have the same effect (Table 1, Expt. 2). ATP, ADP, and AMP were inhibitory whilst

Table 1. The effect of adenine nucleotides and related compounds on the transport of L-alanine into the soluble amino acid pool of isolated calf thymus nuclei. Results expressed in cpm.

Experiment	Addition	cpm	SE	Change, %
	None	8266	±86	0
1	2 mM ATP	5134	187	−38
	2 mM CTP	7748	122	− 6
	2 mM GTP	8362	69	+ 1
	2 mM UTP	7956	203	− 4
	2 mM ITP	8708	133	+ 5
	None	6358	159	0
2	2 mM AMP	4109	136	−35
	2 mM CMP	5816	73	− 8
	2 mM GMP	6109	171	− 4
	2 mM UMP	6289	87	− 1
	2 mM IMP	6148	133	− 3
	None	4821	84	0
3	1 mM ATP	3644	73	−24
	1 mM ADP	3560	71	−26
	1 mM AMP	3779	94	−22
	1 mM adenosine	5014	258	+ 4
	1 mM adenine	4933	65	+ 2
	1 mM phosphate	4874	103	+ 1
	None	4375	66	0
4	5 mM ATP	2927	83	−33
	1 mM ATP	2753	146	−37
	0.25 mM ATP	2989	82	−32
	0.06 mM ATP	3632	61	−17
	1 mM AMP	2821	124	−36
	0.25 mM AMP	3567	75	−18

The nuclei were isolated from thymuses of 2−7 day-old calves, using the procedure described by Allfrey *et al.*[1] 60 mg nuclei (wet weight) were incubated in a final volume of 2 ml containing 125 mM sucrose, 20 mM glucose, 25 mM tris chloride pH 7.1, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 80 mM NaCl, and 5 mM KCl. 25 μg of the transported compound, in this case L-alanine, was present in ^{14}C-labeled form. The mixture was incubated 10 min at 37°C and the reaction stopped by adding to the mixture 20 ml of icecold isolation medium (0.25 M sucrose + 3 mM $CaCl_2$) and sedimenting the nuclei at 750 *g* × 7 min. An extract was immediately prepared from the pellet with 5 ml of 2 % w/v perchloric acid. The debris was centrifuged down and an aliquot of the extract was counted in a Packard Tri-Carb liquid scintillation spectrometer. Routinely 32 incubations were carried out simultaneously, 4 parallel incubations for each experimental value. The averages are given in the tables with calculated *standard errors of means*.

adenosine, adenine, and phosphate were without effect (Table 1, Expt. 3). The concentration of ATP could be diminished to 0.25 mM, the degree of inhibition remaining the same. Lower concentrations caused less inhibition. With AMP the lowest concentration needed to induce the inhibition to its full extent was about 1 mM (Table 1, Expt. 4).

D-Alanine was taken up more than ten times slower than the corresponding natural isomer. L-Arginine was transported at about the same rate as L-alanine and the transport was likewise inhibited by 1 mM ATP (Table 2).

Table 2. The effect of 1 mM ATP on the transport of different amino acids into the acid extractable pool of nuclei.

Compound	Control	SE	1 mM ATP	SE	Change, %
L-Alanine	50 832	918	26 637	1224	−48
D-Alanine	4 037	964	3 704	625	− 8
L-Arginine	54 251	1307	33 672	1529	−38

Experimental conditions as in Table 1.

Amino acids can penetrate the nuclear membrane by two means: physical diffusion and specific transport mechanisms. The apparent K_m for the transport mechanism of L-alanine is 0.8 mM. At a concentration of 0.14 mM the physical diffusion accounts only for 10 % of the total transport, whereas at a concentration of 20 mM diffusion is responsible for the major part, about 80 % (Fig. 1). To see if the physical diffusion was affected by adenine nucleotides, experiments were made using the above-mentioned concentrations of L-alanine. As can be seen from the results in Table 3 the small amount of inhibition observed at the higher concentration of L-alanine can be explained by the inhibition of the specific transport mechanism, the physical diffusion remaining unaffected.

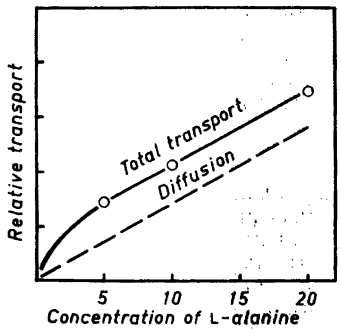

Fig. 1. The concentration dependence of L-alanine transport.

It was thought that Ca^{2+} present in the isolation medium might be responsible for the effect. Similar experiments were made with Mg^{2+} replacing Ca^{2+}. In these experiments the inhibition was equally apparent. When Ca^{2+} was restored to part of the nuclei isolated in a Mg^{2+} medium, it did not cause any change in the transport of L-alanine.

Table 3. The effect of 1 mM ATP on transport of L-alanine at different concentrations.

Concentration of L-alanine	Control	SE	1 mM ATP	SE	Change, %
0.14 mM	7 533	91	4 247	104	−44
20 mM	140 694	1556	121 610	1823	−13

The inhibition induced by ATP is irreversible and persists after pretreatment with ATP at 37°C followed by a brief washing in cold isolation medium. If the pretreatment is carried out at 20°C there is no inhibition (Table 4).

Table 4. Effect of pretreatment with 1 mM ATP at 37°C and 20°C on the subsequent transport of L-alanine at 37°C.

Pretreatment temperature	Control	SE	1 mM ATP	SE
37°C	4016	103	2873	68
20°C	4155	127	4226	145

The nuclei were incubated at the temperature indicated, as described for transport in legend to Table 1 ± 1 mM ATP, but the transported substance had been omitted. The nuclei were washed and resuspended in normal incubation medium with 25 μg of ^{14}C-L-alanine, but no ATP. The rest of the procedure was carried out as in Table 1.

Calf thymus nuclei prepared in these conditions contain ATP in amounts comparable with those in the cytoplasm.

The intranuclear adenine nucleotides do not exchange with externally added carbon-labelled ATP or AMP. The less polar adenosine and adenine, on the other hand, penetrate the membrane easily.[3] The concentrations of adenine nucleotides needed to cause the observed inhibition in transport of amino acids are small and as the nucleotides cannot penetrate the membrane, they probably act directly on it. It seems unlikely that the inhibition is caused by hydrolysis of ATP, because it is brought about by AMP as well. The mitochondria still adhering to some nuclei are uncoupled, owing to the presence of Ca^{2+} and thus unable to form ATP from AMP.

That the inhibition observed is a consequence of a gross physical phenomenon, such as disruption or shrinking of nuclei, seems improbable, as the diffusion of alanine remains unchanged. The fact that the inhibition is not reversed by a brief washing of the nuclei could be explained by a firm binding of adenine nucleotides to the membrane. It is possible that adenine nucleotides are essential for the maintenance of the normal structure and function of the nuclear membrane. During the lengthy isolation procedure the membrane-bound nucleotides may be detached. When during the incubation the adenine nucleotides are again bound to their specific sites in the membrane its configuration returns to the original with resulting changes in the operation of transport mechanisms.

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