Results. As can be seen from the Table, the mouse Crocker sarcoma implanted into the young untreated rats grows only to a small size and regresses quickly. 10 days after the implantation, the tumour is no longer palpable. Heterologous tumour graft rejection follows the same laws as homograft rejection of normal tissue, so well analysed by MEDAWAR[6]. When the rats were *pre*treated with the methylhydrazine derivative, the heterologous tumours grew fast and reached a very large size. These tumours reach their maximal size in an average of 16 days after the implantation and regress only afterwards. The development of the heterologous neoplasms implanted in rats which were treated with I only *after* the implantation, is, however, not influenced by the treatment. These tumours grow only to a minimal extent and complete regression is accomplished already in an average of 9 days after implantation.

Discussion. These results show that, by pretreatment with the above-mentioned hydrazine compound, the immune response against the tissue of a foreign species, in this case the heterologous tumour, is markedly suppressed. The heterograft rejection is retarded. The pretreatment with I elicits the same effect as cortisone, whole body irradiation, amethopterin and mercaptopurine, which are well known to suppress homograft and heterograft rejection of normal and neoplastic tissue[4,7–13]. When the treatment with this compound is only started after the implantation, no suppression of the immunological reaction can be observed. The tumours grow to a minimal size and regress quickly in the same way as the controls do. It seems that a certain time of treatment before the implantation is necessary for the inhibition of the antibody forming system. It could be argued that a certain inhibition of the tumour is brought about by the administration even of small doses of the cytotoxic agent, when the compound is given after the implantation. But this cannot play a role, because we could show in further experiments, that pretreatment followed by continuous treatment after implantation leads to a considerable growth of the heterologous tumours. The abolition of the immune response against the heterograft seems quantitatively to be more important for the growth of these tumours than the direct cytotoxic effect on the tumour.

These experiments add a further new group of compounds to the list of factors capable of suppressing immunological reactions, respectively antibody formation. The methylhydrazine derivative which has been used for these experiments has a depressing effect on certain mesenchymal tissues, as have the other listed measures mentioned above. This gives further support for a relationship between the lymphocytic, plasmocytic and histiocytic tissues on one side, and the antibody formation on the other side. Very probably also other immunological reactions will be depressed.

Zusammenfassung. 1-Methyl-2-*p*-(isopropylcarbamoyl)-benzyl-hydrazin-hydrochlorid (I), ein Cytostaticum, hemmt die Immunreaktion gegen heterologe Tumortransplantate. Diese Reaktion wird nur beeinträchtigt, wenn das Methylhydrazin-Derivat vor der Implantation gegeben wird. Die erwähnte Verbindung gehört einer Gruppe von neuen Cytostatica an, die unter anderem auch die Lymphopoese beeinflussen. Die Zusammenhänge zwischen der Wachstumshemmung gewisser mesenchymaler Gewebe (Lymphocyten, Plasmazellen etc.), der Beeinträchtigung der Antikörperproduktion bzw. der Unterdrückung der Immunreaktionen gegenüber fremden Geweben, werden diskutiert.

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The Changes of the Pentose Phosphate Pathway in Red Cells during their Ageing *in vitro*

It was shown by LÖHR, WALLER et al.[1] that during the ageing of red blood cells (RBC) *in vitro*, gradual decrease of glucose-6-phosphate dehydrogenase (G6PD) can be demonstrated. In spite of having quantitative data about the enzyme activity in hemolysates of the ageing RBC, it is necessary to get some information about the quantitative changes of the production of some intermediates, which occur in the reactions catalysed by this and following enzymes, after RBC is incubated with glucose. Since the activity of this enzyme in normal RBC is limited by the insufficient regeneration of reduced triphosphopyridinenucleotide (TPNH), and since many factors are known to determine the speed of the reaction catalysed by this enzyme[2], it was felt desirable to extend these observations to the investigation of pentose production from glucose during the cell ageing *in vitro*.

50 ml of venous blood was put in the standard acid-citrate-dextrose (ACD) anticoagulant solution and stored for 7 days at room temperature. Immediately after the mixing of the withdrawn blood, and also the 3rd, 5th and 7th day of ageing *in vitro*, 0.5 ml of the packed RBC obtained by centrifugation after the buffy layer is discarded, was put in 1 ml of equal parts of isotonic saline and Na, K phosphate buffer pH 7.4, in which D-glucose was dissolved up to the final concentration 1.2 mg/ml. The suspension was mixed vigorously and aerated, and the amount of glucose decrease and pentose increase during the 3 h incubation at 37° was measured in 10% trichloracetic acid deproteinates by our modification of Bial's orcinol method[3] for the parallel determination of pentose and glucose. Simultaneously, the incubation procedure was made in the medium, where methylene blue was present (final concentration 0.014 mg/ml).

During the RBC ageing *in vitro*, after the mild increase in the first 2 days, gradual decrease of total pentoses was

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observed (Figure 1). When the RBC were incubated with glucose, the pentose production after the 3 h incubation in the fresh blood in average of 12 cases observed was 0.156 μM/ml of the incubation mixture, and diminished gradually during ageing. On the 7th day, when no G6PD activity in RBC stored under these conditions, is measurable[1], the pentose production is between the values 0–0.04 μM/ml (Figure 2). The following factors could be responsible: (1) The fall of initial phosphorylation of glucose due to the ATP decrease[1] and probably to the changes in pH of the stored blood[4], (2) the decrease of G6PD and glucono-6-P-D activity[1], and the subsequent enzymes, which realize the TPNH regeneration. If we correlate the rate of glucose decrease after the incubation of ageing cells to the changes of pentose production from glucose (Figure 3), we have ascertained that the decrease of pentose synthesis is higher than corresponds to the decrease of the glucose utilization. So we can conclude that the decrease of G6PD activity is more distinct than the decrease of the other enzymes which determine the glycolytic activity of the cell, especially the decrease of the initial phosphorylation of glucose in the hexokinase reaction. Since the pentose production in RBC is limited by insufficient regeneration of TPNH, methylene blue, which specifically stimulates the two oxidative steps of pentose phosphate pathway by providing TPNH reoxidation[5], was included in the incubation mixture. Marked stimulative effect in the fresh blood is demonstrable (Table), the pentose production after the 3 h incubation amounts to 35–40% of the utilized glucose (calculations made according to Figure 3).

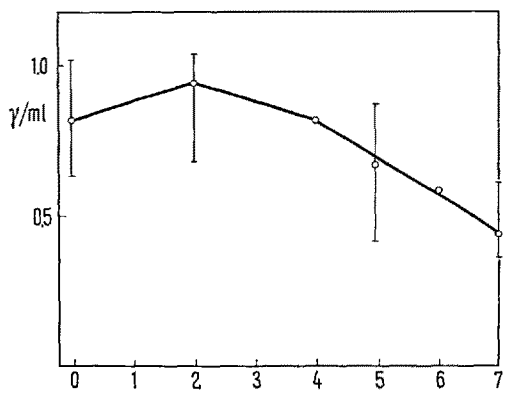

Fig. 1. The pentose values in non-incubated red cells during their ageing *in vitro*. Expressed in μM/ml of the incubation mixture. Abscissa: the age of cells in days, ordinate: the pentose values. Average values of 9 observations.

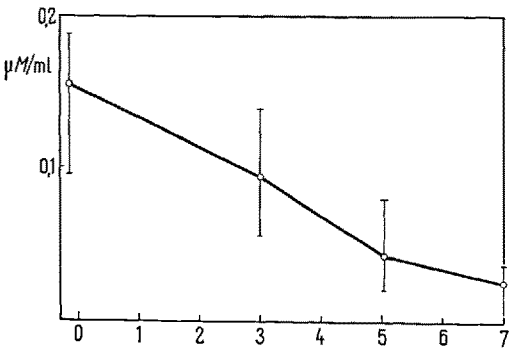

Fig. 2. The pentose production after incubation with glucose in red cells during their ageing *in vitro*. Abscissa: the age of cells in days, ordinate: μM of pentose increase during the incubation with glucose in 1 ml of the incubate. Average values of 9 observations.

During the ageing of the cell, gradual decrease of this effect was seen. On the 7th day of ageing, no considerable stimulative influence of the dye was measurable (Table), the pentose production from glucose is the same as in the incubation mixture without methylene blue. So, the decrease of pentose production from glucose in incubates of RBC is in close relation to the G6PD decrease in hemolysates of these cells. My results are especially interesting in that they differ from the investigations of PRANKERD[6], who observed the production of $^{14}CO_2$ from ^{14}C-1 labelled glucose in RBC to be increased, in spite of G6PD activity in these cells being diminished.

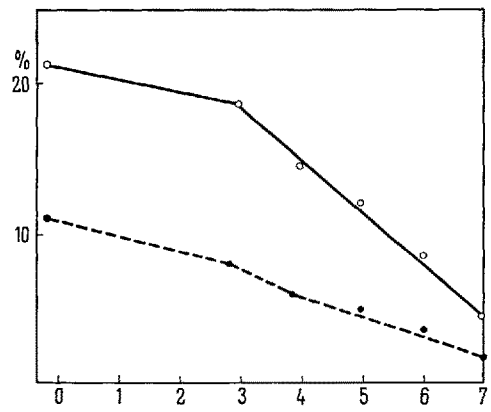

Fig. 3 Glucose utilization and the amount of glucose, transformed to pentose in red cells during their ageing *in vitro*. —— the amount of glucose, utilized during the incubation, expressed in % of the initial value. --- the amount of glucose, transformed to pentose, expressed as the ratio:

$$\frac{\text{pentose increase after 3 h incubation in } \mu M}{\text{glucose decrease after 3 h incubation in } \mu M} \times 100$$

Abscissa: the age of cells in days. Average values of 9 observations.

The stimulative effect of methylene blue on the pentose production during the incubation of red cells with glucose. The results are expressed as the difference between the pentose production from glucose in the presence of methylene blue and the pentose production from glucose without methylene blue. Average values of 9 observations

The age of red cells *in vitro*	The difference in the pentose production in μM/ml of the incubate
0	0.27 μM/ml (0.21–0.30)
3	0.16 (0.13–0.22)
5	0.11 (0.04–0.16)
7 days	0.04 (0.00–0.06)

Zusammenfassung. Im *in vitro* alternden Blut wird Abnahme der gesamten Pentosemenge sowie Abnahme deren Bildung während der Inkubation mit Glucose festgestellt. Das Absinken ist grösser als die gleichzeitige Abnahme der Glucoseauswertung. Ebenfalls nimmt die stimulatorische Wirkung des Methylenblaus auf die Pentosebildung während der Alterung des Blutes stark ab.

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