of isolated E- and M-cells ought to have been considered more carefully by him.

A new investigation of some of these problems seemed necessary: a particular object of the research consisted in determining whether the E- and M-cells behave in the same way with regard to the origin of the comb-rows.

Eggs of *Beroë forskalij*, *Bolina hydatina* and *Eucharis multicornis* were used.

The eggs were freed from their jelly, and at the 8-cells stage the blastomeres were isolated and allowed to develop until the larval stage.

Results. The results obtained were as follows: (1) *Development of one E.* Every E-cell isolated (19 cases) always gives rise to a larva with combs. The combs are arranged in 2 short rows of 6–7 plates each. (2) *Development of one M.* Every isolated M-cell (16 cases) develops into a round, disorganized cellular group, lacking plates. (3) *Development of two E.* Two E-cells (8 cases) give rise to a round larva, smaller than the control, with 4 rows of combs. (4) *Development of two M.* From two M-cells (5 cases) one obtains only a round mass of rather big cells, not organized and always lacking plates. (5) *Development of one E, from which its micromere e_1 was removed.* The resulting larva (9 cases) is very rudimentary, round and completely lacks plates.

From these results one can conclude that only the E-cells of the 8-cell stage have the potentiality for the formation of the combs. This potentiality becomes restricted to the 4 micromeres e_1 deriving from them, at the 16-cell stage.

The 4 micromeres m_1 have nothing to do with the plates. The green plasma which is present in all the 8 micromeres at the 16-cell stage is not responsible for the formation of the swimming plates.

Riassunto. È stato studiato il comportamento dei diversi blastomeri dell'uovo di Ctenofori nei riguardi della formazione delle 8 costole di palette.

Allo stadio 8, la capacità a dare origine alle palette non è ripartita in modo uguale negli 8 blastomeri. I 4 blastomeri E danno origine ciascuno a 2 costole di palette; i 4 M non sono invece responsabili in alcun modo della loro formazione.

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Suppression of the Immunological Reaction by Methylhydrazines, a New Class of Antitumour Agents

In a recent paper we have described the tumour-inhibiting properties of methylhydrazine derivatives[1]. The growth-inhibiting effect is not restricted to tumours; other rapidly proliferating tissues are affected as well. In their haematological and histological investigations, SCHÄRER and THEISS[2] found a lymphopenia and an involution of the germinal centres of the spleen in rats treated with methylhydrazine compounds. It is generally assumed that the reticulo-endothelial and lympho-plasmocytic elements are in some way involved in the formation of antibodies. A series of alkylating agents and antimetabolites, having a pronounced effect on the growth of these tissues, are able to suppress immunological reactions (see review article[3]).

The present experiments were intended to provide information on the effect of the methylhydrazines, a new class of cytotoxic agents, on immunological reactions.

As a test model we chose the immune response against tumour heterografts. Without conditioning the host, an implant of the mouse Crocker sarcoma 180 in young rats grows to a minimal size only and is already necrotized after 6 to 10 days. By conditioning the rats with whole body irradiation or with cortisone treatment, this heterograft rejection is retarded[4]. The tumours grow to a large size and start to regress only after 2 to 3 weeks. The same phenomenon can be brought about by pretreating the rats with a methylhydrazine derivative.

Methods. Young albino rats weighing 40–50 g at the time of tumour implantation were used. The mouse Crocker sarcoma 180 was transplanted subcutaneously as small tumour fragments of 3–5 mm³ size into these rats. Two similar experiments were carried out. In each case 30 animals, being implanted with fragments of the same tumour, were divided into groups of ten rats. One group of rats was not treated and served as control. Ten rats received 8 daily intraperitoneal injections of the methylhydrazine derivative 1-methyl-2-*p*-(isopropylcarbamoyl)-benzyl-hydrazine hydrochloride (I)[5], within the 10 days *prior* to the heterologous tumour transplantation. Daily doses of 50 mg/kg I dissolved in 0.1 ml distilled water were given. Ten rats were injected with the same daily doses of 50 mg/kg, medication starting only *on* the day of implantation. 8 injections within the first 10 days after implantation were administered to the rats of this group. The volume of the well palpable tumours was determined daily; it was calculated with the formula $4/3\ ab^2\pi$ (rotational ellipsoid), a and b being a half of the 2 diameters measured with calipers. Mean tumour volumes are indicated in the Table.

Heterologous transplantation of *mouse* sarcoma 180 in *rats*

Days after implantation	Mean tumour volume in mm³ (each value being an average of 20 tumours)		
	Controls	Treatment with I *before* implantation	Treatment with I *after* implantation
7	1166	2300	825
8	804	3325	416
9	310	4801	0
10	0	7330	0
13	0	7919	0
14	0	9913	0
15	0	10049	0
16	0	10722	0

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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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