activity for sephadex fraction alone and sephadex fraction plus pyridoxal phosphate are nearly the same indicates that coenzyme is not separated from the apoenzyme. On the other hand, the fact that crude extract has less enzyme activity than sephadex fraction may be attributed to the presence of endogenous substrate, product or inhibitors. This assumption is more true in the case of inhibition caused by the presence of the product, since the platelets are known to contain serotonin[2,3].

In the same Table the data of sephadex fraction plus pyridoxal phosphate plus ferric ions, a known activator of 'aromatic amino acid decarboxylase'[17] are shown. The fact that the value of enzyme activity in this case is about the same as those of the other fractions mentioned above, denotes either that ferric ions are not required for the decarboxylation in this enzymic reaction, or are attached to the apoenzyme. For a further explanation of this problem, an attempt was made to check the existence or not of ferric ions in the protein fraction. Thus a portion of sephadex fraction was tested for ferric ion concentration, which was found to be half that of crude extract. The instrument used was the Perkin-Elmer mod. 303 atomic absorption spectrophotometer.

Discussion. The activity of 5-HTP-decarboxylase in human and beef blood platelets and red cells was measured by radiochemical techniques. The relatively large amount of 5-HTP-decarboxylase found suggests the existence of a metabolic pathway for the production of serotonin inside the blood platelets and red cells. Up to now, it was believed that no 5-HTP-decarboxylase exists in the platelets[7] and that these blood constituents were only a storage site for serotonin[4,5]. On the contrary, the existence of serotonin producing enzyme was expected at least for human platelets, since recently LOVENBERG et al.[9] have shown significant activity of tryptophan hydroxylase in human platelets.

The properties of 5-HTP-decarboxylase studied in beef platelets are in accordance with the findings of earlier investigators. Thus the finding that coenzyme is not separated from the apoenzyme, after treatment with sephadex, which suggests that the coenzyme is tightly bound on the apoenzyme, has also been reported by CLARK et al.[7]. From our data, one could not explain whether the ferric ions found in the protein fraction are attached to the apoenzyme or belong to another protein of sephadex fraction. Consequently the main problem, of whether 5-HTP-decarboxylase has any requirement or not for ferric ions, will remain unexplained until extensive purification of the enzyme is obtained. Previous work on this subject has led to contradictory results. Thus LOVENBERG et al.[17] have shown no metal requirement for the decarboxylation of 'aromatic amino acid decarboxylase', while SEKERIS[18], working on the blowfly *Calliphora erythrocephala*, has found a full dependence of a sephadex fraction containing DOPA decarboxylase on ferric ions, while other metallic ions such as Zn, Mg, Mn cannot substitute them.

From the physiological point of view, platelets' serotonin may be involved in the coagulation of blood[19], or may interfere in certain blood diseases, such as thrombocytopenia. The fact that platelets, except their function in known mechanisms, namely uptake storage and release, also contain the biosynthetic mechanism of serotonin, may denote that all these mechanisms help one another to perform the above-mentioned physiological roles, or any others yet unknown. Referring now to the red cells, we cannot express any opinion about the physiological role of serotonin from the present data. Perhaps it may be related to the platelets' serotonin, or may have any other(s) special role(s). This subject apparently needs a further investigation.

Zusammenfassung. Throbozyten aus Menschen- und Rinderblut enthalten eine hohe Aktivität an 5-Hydroxytryptophan-Decarboxylase und für diese enzymatische Reaktion scheint Pyridoxalphosphat als Cofaktor eine Rolle zu spielen.

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The Allergic Macrophage of Graft Versus Host Disease

Graft versus host disease (GVH) is a progressive and often fatal disorder following the injection of incompatible lymphoid cells into hosts which are unable to reject them. Failure of rejection is due either to immaturity of the host, immunosuppression or genetic factors. When parental lymphoid cells of an inbred mouse strain are injected into first generation hybrids, the cells colonise the lymph nodes and spleen which become enlarged. Subsequently the animals lose weight and develop diarrhoea and dermatitis. Autopsy shows lymphoid atrophy, ulceration of the gut and round celled infiltrations of the liver. The pathogenesis of this disease has not yet been elucidated although it is clear that interaction of the injected lymphoid cells with host tissue must play an important part. This report draws attention to an altered reactivity of macrophages in this disease. Macrophages from affected animals are autoaggressive towards lymphocytes and cell death occurs on contact.

Materials and methods. General plan of experiments: GVH was produced by injecting lymphoid cells from donor mice into F × 1 hybrid recipient mice. The treated animals are subsequently referred to as runts. Cell culture monolayers of peritoneal macrophages were prepared from donor, recipient and runt mice. Lymphoid cells from donor, recipient and runt animals were added to these monolayers. The degree of interaction was judged by microscopic examination of stained preparations.

Animals. Donor and recipient strains were C57Bl/6J and B6AF1/J (offspring of C57Bl/6J males and A/J females) obtained from Jackson Laboratories, Bar Harbour, Maine.

Preparation of lymphoid cells. Lymph nodes and spleens were removed aseptically, cut in small fragments and very gently dissociated in tissue culture medium 199 (Glaxo) using a loose fitting hand operated glass homogeniser. Tissue fragments were allowed to sediment and the cells in the supernatant were washed once and

suspended in medium 199 at a concentration of 50×10^6 per ml. Viability as determined by the trypan blue test was 90–95%. A dose of 5×10^6 cells was added to the monolayers of macrophages.

Preparation of macrophage monolayers. Peritoneal cells were sown at a concentration of 1×10^6 cells/ml in test tubes containing rectangular coverslips. Medium 199 containing 10% mouse serum was used. Coverslips were fixed in methanol and stained by Giemsa's method.

Induction of GVH. Parental strain lymphoid cells were injected by the i.v. route into adult F × 1 hybrids. A total of $250–300 \times 10^6$ cells was given in 2 doses at an interval of 24 h. The animals began to lose weight by 21 days and their cells were harvested at 24 days when early signs of illness were apparent.

Results. Cytotoxicity of runt macrophages. Macrophages from runted animals, grown in normal serum, were reacted with normal recipient lymphocytes. The coverslips showed dead cells or fragments of cells (Figure 1). Lymphocytes of donor origin were not affected (Figure 2 and Table).

Cytotoxicity of runt lymphocytes. Although these cells regularly produced cytotoxic effects on monolayers of recipient macrophages, a similar action was noted on monolayers of donor and runt macrophages. Normal lymphocytes added in the same dose to similar monolayers produced no cytopathic effects.

Protective effect of runt serum. Runt serum blocked the cytotoxic effect of runt lymphocytes on normal recipient monolayers and converted the pattern of interaction from cytolysis to adherence. Runt serum had a similar effect on the interaction between runt macrophages and recipient lymphocytes. This serum reacted specifically with recipient lymphocytes and caused them to adhere firmly to monolayers of normal macrophages from either recipients or donors (Figure 3). It had no effect on either donor lymphocytes or lymphocytes from another strain of mouse.

Discussion. It is already known that the reticuloendothelial system (RES) is stimulated by GVH since animals with this disease show an enhanced capacity to clear colloidal particles from the blood (HOWARD[1], DI LUZIO[2]). Our results further implicate the RES in this disorder by showing that macrophages taken from runts are auto-aggressive. When these cells are mixed with normal syngeneic lymphocytes they cause massive adherence and necrosis. This occurs in the presence of normal serum and is clearly a cellular phenomenon. The reaction is highly specific and is not given by lymphocytes from the donor mice of another strain. Although macrophages

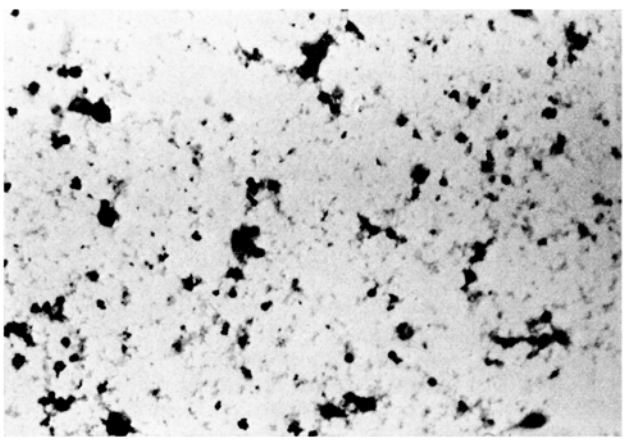

Fig. 1. Normal recipient lymphocytes have interacted with runt macrophages. Only dead cells and fragments of cells remain. Giemsa, × 500.

Source of macrophage	Source of serum	Source of lymphocytes	Result
Specific cytotoxic effect produced by interaction of 'immune' macrophages and normal lymphocytes			
Runt	Normal	Recipient	Cytotoxicity
Runt	Normal	Donor	Nil
Capacity of runt serum to cause adherence between recipient lymphocytes and recipient macrophages			
Recipient	Runt	Recipient	Adherence
Recipient	Normal	Recipient	Nil
Capacity of runt serum to prevent the cytotoxic effect of runt lymphocytes			
Recipient	Normal	Runt	Cytotoxicity
Recipient	Runt	Runt	Adherence
Runt	Runt	Recipient	Adherence

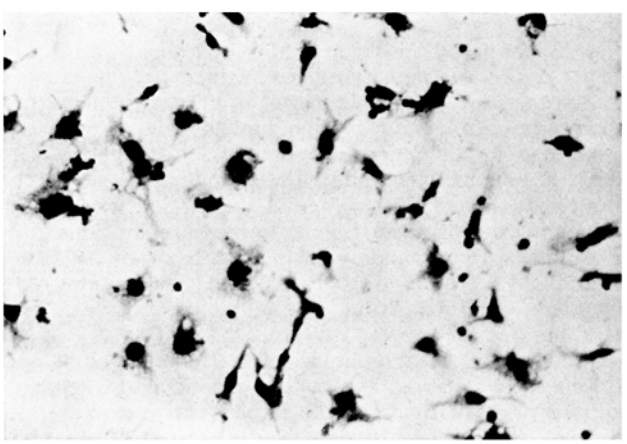

Fig. 2. Normal donor lymphocytes have been added to runt macrophages. Note preservation of cells. Giemsa, × 500.

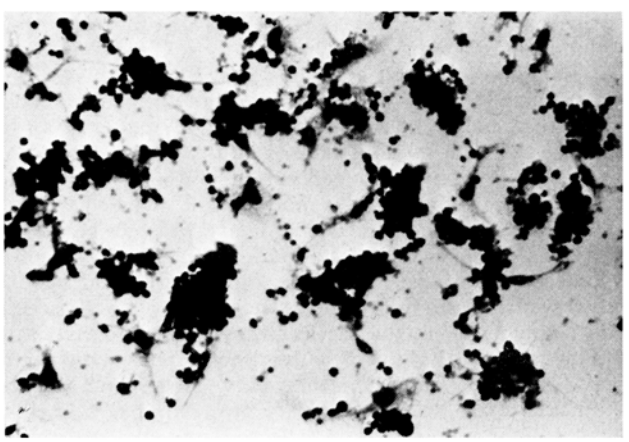

Fig. 3. Agglutination and adherence of lymphocytes around macrophages caused by runt serum. Giemsa, × 500.

are damaged in these reactions we have no convincing evidence that a similar fate is shared by the lymphocyte.

Runt lymphocytes were regularly cytotoxic to monolayers composed of either runt or normal syngeneic macrophages. This is to be expected since many of the lymphocytes are of donor origin and have been stimulated or sensitised by contact with host tissue. These cells were cytotoxic although perhaps to a lesser degree to monolayers of donor macrophages. Since runt lymphocytes and donor macrophages are most probably syngeneic, any form of specific immunological reaction is unlikely. The explanation may be that stimulated lymphocytes exert a non-specific cytotoxic effect on cell lines in culture (LUNDGREN and MÖLLER[3]).

The runt serum contained an anti-lymphocytic antibody which reacted only with recipient lymphocytes. It caused compact adherence of these cells to macrophage monolayers. Its presence blocked the cytotoxic reaction which occurred when runt macrophages reacted with normal lymphocytes. It is noteworthy that in these instances a cytolytic necrotizing reaction was replaced by one of adherence in which numerous well-preserved macrophages were surrounded by rosettes of lymphocytes. Receptor sites, capable of interaction with the modified surface of the runt macrophage, presumably have been blocked by an antibody in runt serum. This phenomenon may be related to the prolongation of homograft survival in vivo noted by FRENCH and BATCHELOR[4]. Whatever the true explanation our findings give to the macrophage a new role in the pathogenesis of GVH, namely an altered reactivity characterized by cytolytic and adherence reactions on contact with 'self' constituents such as lymphocytes[5].

Résumé. Les macrophages péritoneaux prélevés chez des souris au cours de la réaction du greffon contre l'hôte (RGCH) ont été mis en culture sur des lamelles. Ces cellules sont caractérisées par une plus grande variabilité de taille, une activité de membrane plus prononcée (observée au microscope à contraste de phase) et une avidité plus grande envers des erythrocytes sensibilisés. Le fait principal est que les macrophages des souris RGCH sont auto-agressifs quand ils sont mélangés avec des lymphocytes normaux de souris syngénéiques.

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Soluble Antigen-Antibody Complexes Reactive in Homologous Rabbit Skin

Rabbit homologous skin sensitizing antibodies have been elicited experimentally by the injection of proteins[1], hapten conjugated proteins[2,3], or by infection with various nematodes[4]. They are usually detected 6 days after immunization and disappear within a few days or weeks[2]. More recent reports[5,6], however, indicate that skin sensitizing antibodies may persist for longer periods in rabbit serum and that their characteristics are dependent on the type of immunization. Such homocytotropic antibodies are reported as being associated with γG-[3,5], γA-[7] and recently with γE-like immunoglobulins, the latter occurring in a very small quantity in the circulation of the rabbit after immunization[1,8].

As for human reaginic hypersensitivity reactions, evidence has been presented showing that preformed ragweed-γE antibody complexes give erythema-wheal reactions in normal individuals, whereas the complexes composed of either γG or γA antibody do not[9]. The present study was undertaken in order to test the ability of soluble antigen-antibody complexes to elicit immediate-type skin reaction in normal rabbits. Both 'early' and 'late' antibodies were examined in these complexes. The nature of the antibodies involved in the skin reactions was investigated under different physiochemical conditions.

Randomly bred albino rabbits of either sex, weighing 2.5–3 kg, were used for immunization and reverse type skin reactions. 10 rabbits were immunized with 2 ml DNP-BSA (1 mg/ml) emulsified in complete Freund's adjuvant. The immunogen was prepared by the method of EISEN[10], contained an average of 18 DNP molecules per molecule of BSA and was injected at multiple intradermal sites. A booster i.m. injection was administered 10 and 16 days later. The animals were bled on the 6th, 10th, 16th and 30th day after immunization.

The sera from individual bleedings were pooled to produce a 6th day serum, 10th day serum etc. Quantitative precipitin test[11] of these antisera with DNP-ovalbumin revealed 0, 0.3, 1.1 and 1.9 mg/ml respectively of precipitating antibodies. Globulins were separated from immune sera by precipitation at 4 °C with ammonium sulfate (50% saturation); they were then washed twice with 50% saturated ammonium sulfate, dissolved in distilled water and dialyzed against 0.15 *M* NaCl. Reduction and alkylation of globulin samples was done as previously described[3]. Heat treatment were carried out at 56 °C for 7 h in a shaking water-bath. Succinillation of the globulin fractions was done according to RIVAT et al.[12] using 10% succinic anhydride.

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