In the lymph there was no increase in the protein concentration, which must therefore have been retained at the Arthus site. This retention does not occur after a non-immunological inflammatory reaction such as thermal injury in which the plasma protein passes directly into the lymph.

In the lymph pellet (i.e., leucocytes separated by centrifugation) there was an increase starting 4–8 h and reaching a maximum 16–20 h after challenge, in the activities of LDH, β-gluc, cathepsin D and GOT. This increase corresponded to the increasing number of cells leaving the site of injury and entering the lymph.

In the lymph supernatant there was an increase only in LDH, β-gluc and GOT at 16–20 h. At least part of the β-gluc probably originated in the plasma where there is a high level of this enzyme. There was no increase in the activity of cathepsin D in the supernatant although it was increased in the pellet and at the Arthus site. Therefore if the LDH and GOT (and possibly part of the β-gluc) activities originated at the Arthus site from damaged cells it must be postulated that cathepsin D was inactivated in some way. On the other hand, if they originated in leucocytes which had left the site and entered the lymph, it seems likely that there was leakage only of cytoplasmic enzymes and not of lysosomal enzymes.

A new approach for studying the influence of cyclophosphamide upon the rejection of rabbit skin homografts

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Previously, Bitterli & Jasani (1972) showed that during the homograft reaction changes in dry weight parallel increases in vascularity of the graft, increases in the moisture content reflect changes in vascular permeability and those in the DNA content indicate increases in the cellularity of the graft.

The present experiments show that when the homograft reaction is suppressed the changes of these three parameters are modified such that they resemble the corresponding changes in skin autografts.

When the rabbits received daily intravenous injections of cyclophosphamide, so that the total dose was 745 mg per animal, the reaction was maximally suppressed. In these experiments the tissue dry weight and DNA of the homografts did not increase above the values found in autografts that were transplanted onto similar anatomical sites in the opposite leg of the same animal, and which therefore constituted the internal control. The two types of graft, i.e., homografts and autografts in these animals, also resembled each other in outward appearance and histologically in both epithelial hyperplasia was present and lymphocytic mononuclear cells virtually absent.

In contrast, in rabbits receiving a total dose of cyclophosphamide of 445 mg/animal, rejection was only partially suppressed, i.e., the healthy pink appearance of their homografts did not last for even as long as 24 h beyond the expected time of onset of rejection. Whilst their autografts continued to be incorporated normally into the surrounding skin, the homografts of these animals became bluish purple and firm in consistency, and histologically they developed evidence of mononuclear cell infiltration. In this group the DNA as well as the dry weight of homografts increased to a significantly greater extent than in the paired autografts. However, although the influence of this dose of cyclophosphamide was not detectable using the usual three parameters, there were differences in the histological changes in the grafts. Compared with homografts of the non-treated animals, the degree of epithelial hyperplasia was greater whereas the mononuclear cells were fewer.

Although the estimation of DNA provides an accurate assessment of cellularity, it does not give an indication of changes in the population of different cell types.

Therefore new parameters were necessary to distinguish between hyperplasia of cells normally present in the skin and changes in the number of migratory cells. Earlier

experiments (Jasani & Lewis, 1971) indicated that estimation of changes in lactic dehydrogenase and β-glucuronidase activities may be used to assess the magnitude of increase in the number of lymphocytic cells which are the main migratory cells in the homograft reaction. It is now concluded that acid phosphatase activity can be used as a reliable index of epithelial regeneration, since there is a good correlation between the degree of epithelial hyperplasia observed on histological examination of the grafts and their acid phosphatase content.

REFERENCES

BITTERLI, E. & JASANI, M. K. (1972). A quantitative assessment of tissue changes accompanying homograft reaction: changes in tissue dry weight, DNA and moisture content in rabbit skin homografts. *Br. J. Pharmac.*, **45**, 138–139P.

JASANI, M. K. & LEWIS, G. P. (1971). Lymph flow and changes in intracellular enzymes during rejection of rabbit skin homografts. *J. Physiol. Lond.*, **219**, 525–554.

A pro-inflammatory effect of adrenaline in thermal injury

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Thermal injury induced by immersing the hind paws of rats in water at 46·5° C for 60 min causes the formation of an inflammatory oedema (Rocha e Silva & Antonio, 1960). Whilst studying the anti-inflammatory activity of catecholamines it was found that local injection of adrenaline (0·1–1 μg) or noradrenaline (0·2–1 μg) 15 min prior to heating the hind paws of rats potentiated thermic oedema. This was unexpected, since catecholamines antagonize paw oedema induced by injection of irritants or pro-inflammatory mediators (Green, 1972).

Studies by Rocha e Silva & Antonio (1960); Starr & West (1967) and Rocha e Silva, Garcia Leme & De Souza (1969) have implicated bradykinin as the mediator in thermic oedema. It was therefore of interest to establish whether catecholamines potentiate thermic oedema by enhancing the formation of bradykinin. Heated paws were co-axially perfused either with Tyrode's solution alone or with Tyrode containing graded concentrations of adrenaline. The perfusate was subsequently assayed for bradykinin, kinin-forming activity and kininase activity as described by Starr & West (1967). Rat uterus, rat duodenum and guinea-pig ileum were used as assay preparations, the bathing solutions containing sotalol hydrochloride (10^{-5}–10^{-4} g/ml) and phentolamine hydrochloride (2×10^{-6} g/ml) to antagonize the effects of adrenaline. Perfusate collected over 30 min from heated control paws contained bradykinin 14·1±2·7 ng (S.E. of mean, $n=8$) whereas perfusate from paws perfused with adrenaline (0·5 μg/ml) contained bradykinin 7·6±2·2 ng. Adrenaline had no significant effect on kinin-forming activity but increased kininase activity 76%. These results indicate that the potentiation of thermic oedema by adrenaline is not due to activation of kinins. Furthermore, adrenaline suppressed the increase in vascular permeability induced by injection of bradykinin or kallikrein.

The pro-inflammatory effect of adrenaline was antagonized by pretreatment with phenoxybenzamine (10 mg/kg, I.V.) but not by propranolol (10 mg/kg, I.P.). Like adrenaline, local injection of vasopressin (25 M.U.) was found to potentiate thermic oedema, suggesting that this property may be common to vasoconstrictor agents. A thermistor probe inserted into the paw showed that a temperature gradient of 1·2±0·2° C existed across the skin of control paws heated at 46·5° C compared with a temperature gradient of only 0·1–0·2° C in heated paws injected with adrenaline (1 μg), although both adrenaline-treated and control paws had similar internal temperatures after 30 min of heating. However, even small changes in temperature are of importance in the development of thermic oedema, since it was found that paws heated at 47·5° C for 15 min and then at 46·5° C for 45 min had significantly greater oedema than paws which were heated at 46·5° C for 60 min.

The pro-inflammatory effect of adrenaline in thermic oedema may therefore be attributable to a reduction in blood flow in the injected paws, with the result that heat