meter, solitary or grouped. In some cases the entire aorta was transformed into a stiff tortuous, dilated tube. In the noradrenaline group 3 out of 10 aortae showed clear-cut aneurysmatic dilatations of the proximal segments of the vessel; in the noradrenaline-prednisone group the lesions were evidently more advanced, 6 out 10 animals showing extensive aneurisms frequently reaching the abdominal aorta.

In both groups of animals the main histological changes in the pulmonary artery were represented by accumulations of PAS-stainable intercellular material in the subendothelial spaces and in the innermost layers of the tunica media; in some cases small necrotic foci were seen. Calcifications were never observed. Instead, in the aorta, besides mucoidosis and widespread necroses of the medial smooth muscle, deposition of calcium was a common finding in the necrotic areas of the tunica media as well as more diffusely in the amorphous ground substance in between the elastic membranes. Stretching and splitting of the elastic lamellae at the site of the calcifications and a slight increase in the amount of collagen in the immediate surroundings of the calcified lesions were observed. In agreement with the gross changes, the microscopic changes were more severe and diffuse in the noradrenaline-prednisone group than in the noradrenaline group (Table 1).

Table II shows the results of ^3H thymidine studies. From this Table it appears that long-standing noradrenaline treatment leads to the appearance of ^3H labelled nuclei in the tunica media of both the aorta and the main pulmonary artery. Further, it appears that ^3H thymidine incorporation is significantly increased by the simultaneous administration of prednisone. In both groups of animals the labelling was particularly evident in the surroundings of the necroses and calcifications. In the single cases a rough correlation was found to exist between the severity of the medial lesions and the rate of ^3H-thymidine uptake. From the results reported above it appears that i.v. noradrenaline treatment may induce in the rabbit vascular lesions which are quite similar to those elicited by adrenaline; actually the main changes observed have been necrosis of smooth muscle cells and focal or diffuse deposition of calcium salts[16-18]. Concomitantly, as shown by the autoradiographic study, the uninvolved smooth muscle of the tunica media proliferates in an attempt to repair and restore functional and structural integrity of the arterial wall. In agreement with others, it was found that the noradrenaline-induced arteriopathy is greatly facilitated by the accompanying administration of prednisone[19,20]; besides, under steroid treatment the nuclear DNA synthesis in the medial coat of the arteries was found to be significantly increased. This latter finding was rather unexpected since it is widely taken for granted that glucocorticoids are potent inhibitors of cell reactions and proliferation. Consequently, the enhancing effect of prednisone on the noradrenaline-induced arteriopathy cannot be related to an inhibitory effect on the reparative proliferation of the well-differentiated smooth muscle cells of the media, but more likely to an effect of the glucocorticoid on the extracellular components of the arterial wall[21].

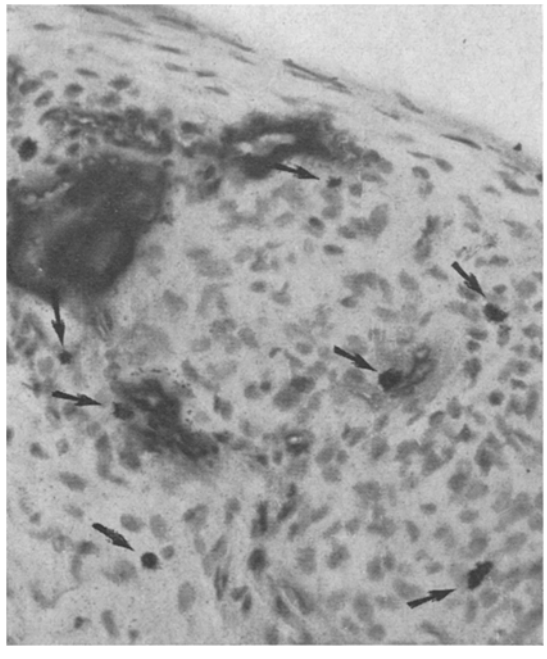

Fig. 2. Aorta of a noradrenaline-prednisone treated rabbit showing numerous labelled nuclei (arrows) in the surroundings of a medial calcified plaque. × 250.

Riassunto. Si è studiata la morfologia e la proliferazione cellulare che si instaura nella media dell'aorta e dell'arteria polmonare di conigli sottoposti a trattamento con noradrenalina e con noradrenalina-prednisone facendo uso del metodo autoradiografico dopo somministrazione di ^3H timidina. Le lesioni e la proliferazione della muscolatura della media che si attuano in seguito a somministrazione di noradrenalina vengono sensibilmente aggravate dal trattamento contemporaneo con prednisone.

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Lymphocyte-Stimulating Activity of Scarlet Fever Toxin

There is some indirect evidence suggesting that the biological activity of scarlet fever toxin is partially dependent on previous sensitization of the organism[1,2]. If this is so, cell hypersensitivity should be demonstrable by the test of blastic transformation of lymphocytes in vitro, which moreover allows distinction – on the basis of the quantitative parameters of the response – between the reaction of the sensitized lymphocyte to a specific antigen and the reaction to nonspecific mitogens.

Scarlet fever (erythrogenic) toxin (ET) was produced in vitro by the group A β-haemolytic streptococcus strain NY Dochez 5 and was prepared and purified essentially

Lymphocyte-stimulating activity of scarlet fever toxin

Donor of cells	Thymidine-^{3}H uptake after ET (STD[a])						PHA (µl)
	3	3×10^1	3×10^2	3×10^3	3×10^4	3×10^5	50
Normal	1.67	3.24	12.4	28.6	24.1		16.8
Tolerant	0.87	0.89	1.39	5.13	21.9	22.1	20.6

[a] Skin test doses.

according to STOCK[3]. The stimulating activity of this preparation was tested on chinchilla rabbit blood lymphocytes and was evaluated in terms of ^{3}H-thymidine uptake. Lymphocytes, obtained from defibrinated blood after sedimentation with 3% gelatin, were suspended to a concentration of 1×10^6 cells/ml in modified Eagle's medium supplemented by 20% of inactivated normal pooled rabbit serum. 2 ml aliquote were cultivated either with varying concentrations of ET, with PHA (positive control) or without any additions (negative control). After 3 days of cultivation at 37 °C in humidified 5% CO_2 atmosphere, 1 µCi of ^{3}H-thymidine/ml was added 5 h before harvesting. Cells were harvested by the filtration method. The trichloroacetic acid (TCA) precipitate was dissolved by overnight incubation with 0.5 ml of 98% formic acid and after evaporation counted (liquid scintillation spectrometer Marck I). The mean uptake values expressed as the ratios of cpm of stimulated cultures to negative control cultures, are presented in the Table.

The lymphocyte response to ET has all the signs of nonspecific stimulation: the cultivation period necessary for the maximum response, the course of the relation between response and stimulant dose and the degree of activation are comparable with the reaction to PHA; reactivity without previous sensitization was demonstrated in all the animals tested. Since the stimulating activity of ET can be neutralized by antitoxin serum (Wellcome Streptococcus Antitoxin–Scarlatina), the effect is apparently due to ET itself; however, the possible share of another active substance accompanying ET cannot be safely excluded. The question offers itself whether there is any relationship between the mitogen demonstrated by us and the active substance in other streptococcus exoproducts, e.g. the streptolysin S preparation[4–7] where the mitogenic activity has proved to be quite distinct from haemolytic activity.

By daily i.v. injections of ET specific tolerance can be induced[1], which is demonstrable even in the lymphocyte response in vitro (Table); the inhibition has a similar dose-dependent character as the pyrogenic tolerance in vivo. The reactivity to PHA is retained. Since ET can activate a much higher percentage of the lymphocyte population than can presumably be involved in the reaction between sensitized lymphocytes and specific antigen, obviously neither can the tolerance be explained in terms of immunological mechanisms. We suggest that the lymphocyte activation is due to ET bondage to specific receptors carried by a large part of the cell population. Tolerance is then occasioned by a temporary blocking of these receptors; the ability to react with other stimulants is retained, as has been shown even in the production of leukocytic pyrogen[8] and the migration inhibition factor[9] in vitro.

The finding that ET is able to activate lymphocytes coextensively with nonspecific mitogen is important in several respects. Primarily, this can contribute to the explanation of some of its biological effects wanting in satisfactory interpretation, e.g. its immunosuppressive effect[10], a feature well-documented in non-specific mitogens, and possibly also the ability to enhance organism sensitivity to the effect of other toxins[1,2]. Another, more general aspect relates to the mechanism of ET action in the organism. After i.d. injection PHA induces a local reaction of delayed type hypersensitivity, and the production of factors of cell-mediated immunity after activation of lymphocytes by nonspecific mitogens has been demonstrated[11,12]. We suppose that even ET may be a substance capable of inducing, by a non-immunological mechanism, the manifestations of immunologically specific activation of lymphocytes. Finally, the metabolic consequences of lymphocyte activation, such as the activation of lysosomes and production of a number of biologically active mediators, provide a real basis for the speculations on the role of ET in streptococcal infections and their sequelae.

Zusammenfassung. Das erythrogene Toxin stimuliert die Inkorporation von ^{3}H-Thymidin in Blutlymphocyten von normalen Kaninchen. Die Reaktion hat den Charakter einer Antwort auf ein nichtspezifisches Mitogen. Die in vivo erzeugte Toleranz führt zu einer Dosis-abhängigen Hemmung dieses Effektes.

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