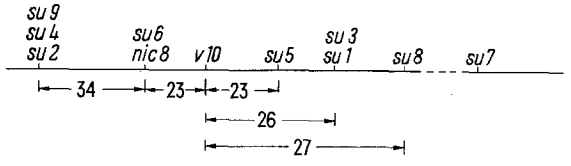
also alleles. Crosses involving *su7* deserve further consideration. In all crosses involving such strain, 'crinkled' colonies were obtained. Similar colonies were obtained by other authors in *A. nidulans*[13,14], and in all cases a duplication was involved. In *su7* case all crinkled colonies were phenotypically *nic*$^+$; it is possible that a duplication is involved which causes the crinkled phenotype and independence to nicotinic acid.

The dominance-recessiveness relationships among the 9 suppressors were carried out by formation of heterokaryons homozygous for *nic8* allele and heterozygous for the suppressor tested (MSE *su nic8* × *su1 ad20, y, ad20; phen2; pyro2; lys5; s3; nic8; ribo2*) and by growing them on MM supplemented with pyridoxine, thiosulfate and riboflavine. In all 9 cases the heterokaryon could grow on this medium. On the other hand, with one exception, diploids obtained from such crosses were unable to grow on the same medium. The exception was the diploid heterozygous for *su6*, probably an intragenic suppressor. These results were taken as experimental support for the hypothesis about the mechanism of gene regulation, which is an adaptation to the 'Cascade regulation mechanism' proposed by PONTECORVO[1]. According to this hypothesis, *nic8* would be a mutant in the regulator R_2 which does not make a repressor active to block the action of the regulator R_1, thus inhibiting the action of the structural gene. In our case, the other loci would be collectively responsible for the syntheiss of the other repressor. Mutation in one or more of these loci would be sufficient to prevent the synthesis of the second repressor, thus enabling the structural gene to synthesise its enzyme. Such repressor (R_1) would be made of several units resulting in a complex chain. Each of these units would be produced by a specific locus, where any mutation results in an absent or altered subunit and, consequently in a non-functional complex. Of course, other explanations could be proposed, as, for instance, an adjustment on MM in favour of 1 nucleus in heterokaryons[3]. On the other hand, the nuclear location of repressors cannot be ruled out and the existence of systems of regulation at nuclear level was already proposed for eucaryotes[15]. All suppressors analysed appear to exhibit striking differences in their gene structures, since they showed difference in growth rates (Table II). Also they exhibit a pleiotropic effect, since most of them conferred at the same time independence to nicotinic acid requirement and resistance to *p*FA (Table III). Such effects can be explained by the fact that some substances including shikimic acid are common precursors of phenylalanine and nicotinic acid[4]. More consistent knowledge of the suppressor genes used by us could however, only be obtained from more detailed genetic and biochemical studies.



Location of the different suppressors of *nic8* allele analysed (linkage group VII). Distance are only approximated and are not in scale on the map.

Zusammenfassung. Neun Suppressoren des Mutanten *nic8* von *Aspergillus nidulans* wurden in der Kopplungsgruppe VII der Chromosomenkarte lokalisiert und unter verschiedenen Aspekten studiert. Eine Hypothese über die Mechanik der genetischen Regulierung der Erzeugung der Nikotinsäure wurde formuliert.

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Effect of Influenza Virus PR8 Infection on Thymus in Intact and Adrenalectomized Mice

The mechanisms of thymus involution are very complex and not well understood. Many external agents responsible for thymus involution instead of acting separately trigger intrinsic mechanisms of the organism, such as the secretion of corticosteroids, which contribute greatly to thymolysis (for ref. see [1]). This laboratory has previously reported[2] a drastic reduction in thymus weight after influenza virus PR8 infection of mice. In the present study we analyzed the extent of thymus reduction caused by virus infection in adrenalectomized mice as compared with thymus damage in intact infected mice. We were also looking for morphological differences in intact and adrenalectomized mice.

Materials and methods. Highly inbred BALB/c mice, 6 weeks old, intact, adrenalectomized[3] and sham adrenalectomized (10 animals per group) were inoculated intranasally with 0.1 ml of influenza virus suspension (PR8, A_0 type, 13 H.A.U./ml). Viral infection was controlled by the presence of serum antibodies detected by haemagglutination inhibition test. Adrenalectomized mice showed higher susceptibility to the virus, but the infection was only rarely fatal. Thymuses of all groups of mice were collected daily, from 1st to 10th day after virus inoculation, after fixation in situ (DJACZENKO and CIMMINO[4]). Tissue was embedded in Vestopal W and semithin sections were stained with 0.1% toluidine blue.

Thymuses presented in Figures 2–5 were prefixed in situ with a mixture of acrolein-glutaraldehyde and TAPO[4], postfixed with 4% osmium tetroxide, dehydrated and embedded in Vestopal W. Semi-thin sections (0.5 μm thick) were stained overnight with 0.1% solution of toluidine blue in 1% borax.

Results. Figure 1 shows that thymuses of infected mice undergo a drastic reduction of weight, more pronounced in intact animals. Morphology of the thymus of normal control mice may be observed in Figure 2. Thymuses of

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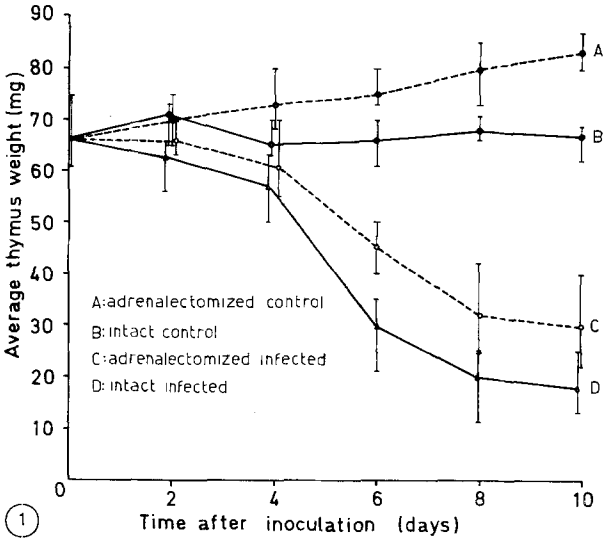

Fig. 1. Distribution of thymus weights in adrenalectomized control (A), intact control (B), adrenalectomized infected (C) and intact infected (D) mice. The weight of thymuses in the group of sham operated animals corresponds to that of intact infected animals. Each group is composed of 10 animals. Reduction of thymus weight in infected mice is evident.

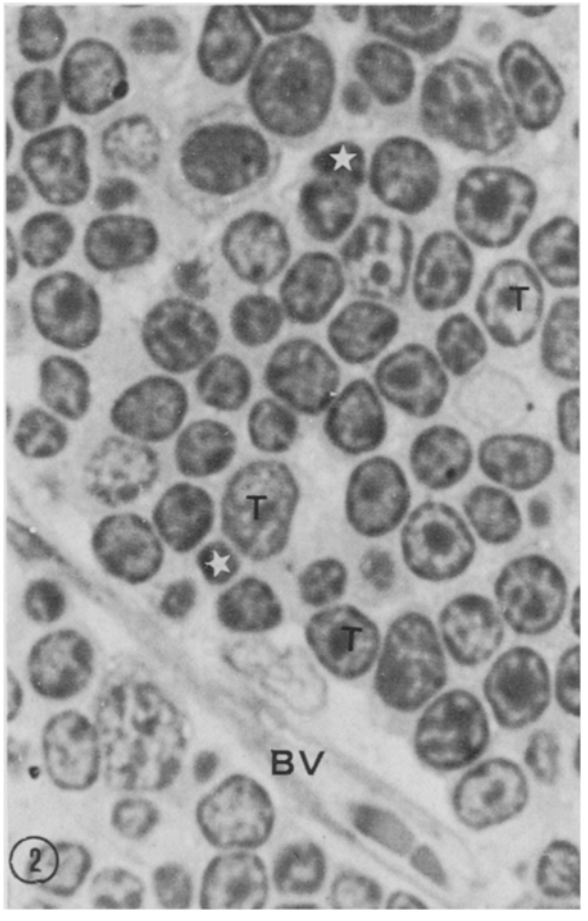

Fig. 2. Light micrograph of the thymus from a normal control mouse. Dark reticular epithelial cells (DREC) are marked by stars. Thymocytes (T) show a normal differentiation of the size and staining quality. A blood vessel (BV) is seen at the bottom of the micrograph. ×1,200.

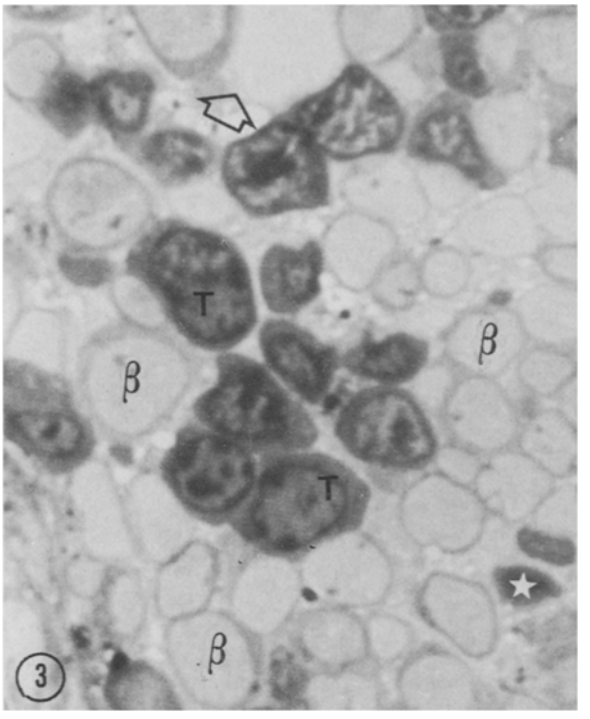

Fig. 3. Thymus of the adrenalectomized mouse collected on 6th day after virus infection. Thymocytes form 2 populations; one with a normal staining capacity is marked 'T' and another scarsely stained is marked 'β'. Focal thymolysis is indicated by an arrow. DREC are marked by a star. ×1,200.

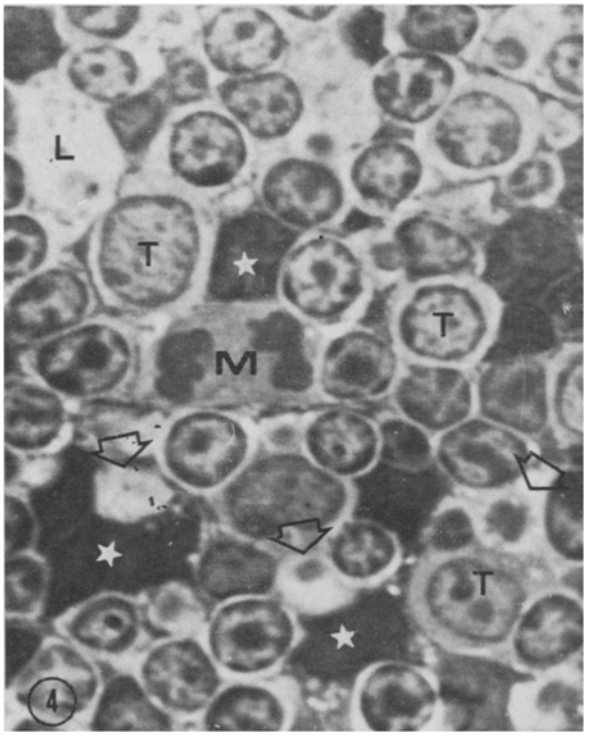

Fig. 4. Thymus of adrenalectomized mouse collected on 10th day after virus infection. Focal thymolyses are indicated by arrows. Damaged DREC are marked by stars. L is a light reticular epithelial cell with clear signs of degeneration. Many of thymocytes (T) show a tendency to bind little stain. Their cytoplasm is optically lucid. A mitosis may be seen in one DREC (M). ×1,200.

adrenalectomized infected mice observed on 6th day after infection (Figure 3) are characterized by the damage of dark reticular epithelial cells (DREC) and focal thymolysis. Moreover cortical thymocytes show a great difference in the intensity of staining (Figure 3). The population of intensely stained thymocytes is less numerous. At a later period of virus infection (10 days, Figure 4), the cortical layer of thymocytes tends to disappear. Focal thymolyses are more frequent. At the same time a number of mitoses may be observed in the thymus (Figure 4).

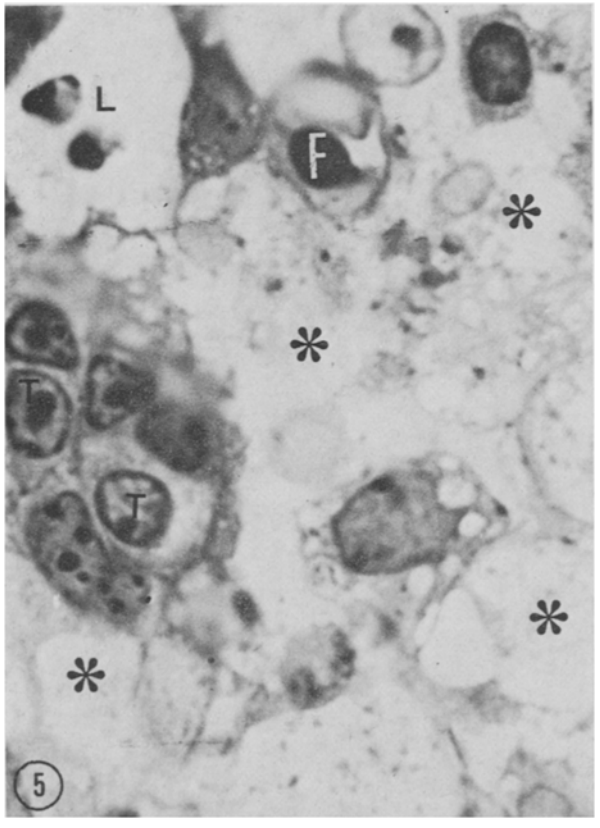

Fig. 5. Thymus of intact infected mouse collected on 10th day after virus infection. Large confluent zones of lysed cells are marked by asterisks. A small islet of fairly well preserved thymocytes (T) is seen on the left of the micrograph. Phagocytized pyknotic nucleus of the thymocyte is marked 'F'. At 'L' a light reticular epithelial cell containing 2 huge lysosomes. ×1,200.

Virus infection of intact mice rapidly causes a complete disappearance of the cortex of the thymus. In the medulla DREC are heavily damaged and a focal thymolysis is a frequent phenomenon. On 10th day after virus inoculation thymuses show large confluent zones of lyzed cells (Figure 5). Only small islets of practically intact cells may be observed (Figure 5). We did not observe any mitotic divisions during the whole period of virus infection of intact mice.

Sham operated infected mice behave in all respects as intact infected mice.

Discussion. PR8 virus infection provokes the thymus involution in mice[2]. We now report the morphological alterations responsible for the involution, which is more clearly expressed in intact mice than in adrenalectomized mice. A model of indirect effects of PR8 virus infection on thymus is offered by adrenalectomized mice. DREC damage is common to both experimental groups of animals. Focal thymolysis is best seen in adrenalectomized mice. We were also able to distinguish, on the basis of the intensity of staining with toluidine blue, two cell types in the population of thymocytes in adrenalectomized infected mice. It is reasonable to advance a hypothesis that less intensely stained thymocytes, not present in intact infected mice, probably correspond to the corticosteroid-sensitive population of thymocytes in the classification of RAFF and CANTOR[5]. For a better functional definition of the less intensely stained thymocytes more experiments are needed. Thymuses of adrenalectomized mice show a tendency to recover at later experimental periods as shown by the presence of mitotic divisions.

Riassunto. Le alterazioni morfologiche del timo nel corso dell'infezione con virus PR8 sono più evidenti nei topi normali che nei topi adrenalectomizzati, i quali mostrano una differenziazione dei timociti in due classi sulla base dell'intensità di colorazione con bleu di toluidina.

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Induced Mutations in *Serratia marcescens* by Near UV-Light in Presence of Psoralen

Ultraviolet light of wave length ranging 253–265 nm results in a rapid inactivation of cells of micro-organisms and consequently yields a low percentage of mutants among survivors. The present study was aimed to find out the effect of near UV-light (NUV) of 350–360 nm wave length on the mutagenesis in *Serratia marcescens* (threonine less mutant). A photosensitizing chemical, 8-methoxy psoralen (MOP) was used to increase the effect of NUV, since at this wave length, irradiation alone was ineffective in cell inactivation (Figure).

Treatment of cells with MOP was done before near UV-irradiation. 8-methoxy psoralen (Manaderm, Jeoffrey Manners Co. Ltd., India) was dissolved in absolute ethyl alcohol to give 1 mg/ml solution. 1 ml of MOP solution was added to 9 ml of the cell suspension of *Serratia marcescens*(threō) grown in DAVIS[1] minimal medium. The cell suspension was left for $^1/_2$ h, in dark for uptake of MOP. The cell suspensions treated with or without (MOP) were subjected to near UV-irradiation for different intervals of time in an open Petridish at a distance of 15 cm from a 125 W UV-lamp (Philips Model 57236 E 170 HPW, Holland). Mutations were scored for 1. reversions to threonine independance; 2. forward mutations to

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