concentrations of urea suggest that both ionic and hydrogen bonding forces are operative. Hydrophobic attractions, which are important in polymer interactions, also may play a significant role in the formation of the phthalanilide–nucleic acid complex.[8]

Yeast RNA and polyadenylic acid also interact with NSC 60339 to produce a bathochromic shift in the absorption maximum of the phthalanilide in the presence of either of these ribose nucleic acid polymers. Unlike the complex observed with DNA, which appears to form a stable complex immediately after addition of phthalanilide, the RNA–phthalanilide complex results in a continuing aggregation of particles, giving rise to a visible Tyndall effect after several minutes. This aggregation increases slowly over a 24-hr period. The DNA–phthalanilide complex, on the other hand, is stable over a 24-hr interval at room temperature.

That the interaction of phthalanilides with both ribose- and deoxyribosenucleic acid polymers is relatively nonspecific is further supported by experiments showing that DNA preparations isolated from either NSC 60339-sensitive or -resistant mouse leukemia cells react with NSC 60339 in essentially the same way as does the salmon-sperm DNA.

Whether the interaction with nucleic acids bears any relationship to the antitumor activity of the basic phthalanilides cannot be determined with certainty at this time. However, it is intriguing to speculate on the possibility that the phthalanilides may mimic the histones in their ability to combine with DNA and thereby possibly repress or completely inhibit certain information transfer mechanisms—i.e. they may possess "anticodic" activity.[2] Although the DNA preparations from resistant and sensitive cells interact with phthalanilides to the same degree under conditions *in vitro*, interactions *in vivo* may differ. In fact, mouse leukemia cells resistant to NSC 60339 retain less than 20 per cent of the amount of phthalanilide bound by sensitive cells after exposure for 24 hr *in vivo*.[9]

Experiments are in progress to clarify the possible importance of the DNA-phthalanilide interaction in inhibiting the growth of mouse leukemia cells.

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## A specific esterase in experimental inflammation

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DURING the investigation of the anti-inflammatory activity of the protease-inhibitor from potatoes (IPP),[1-3] an attempt was made to characterize the hypothetical enzyme involved in the development of inflammation and inhibited by IPP. For this purpose, a peptidic preparation of IPP was used possessing a high anti-inflammatory activity and, with the exception of chymotyrpsin, almost no inhibitory activity against some common proteolytic enzymes (trypsin, plasmin, papain). The hydrolysis of synthetic substrates (tosyl-L-arginin-methyl ester, L-lysin-ethyl ester and acetyl-L-tyrosin ethylester) was studied with a modified photometric indicator method[4] by extracts of inflamed tissue (kaolin-arthritis in rats, paws frozen to −40°, homogenized and extracted with saline). Only acetyl-L-tyrosin ethyl ester (ATEE) was hydrolyzed much more by the extracts from the inflamed tissue than by extracts from the normal tissue or tissue from inhibitor-treated rats (3 mg/kg of IPP i.p., 30 min before the kaolin injection Fig. 1). The hydrolytic activity of the inflamed tissue on ATEE increased in a parallel way to

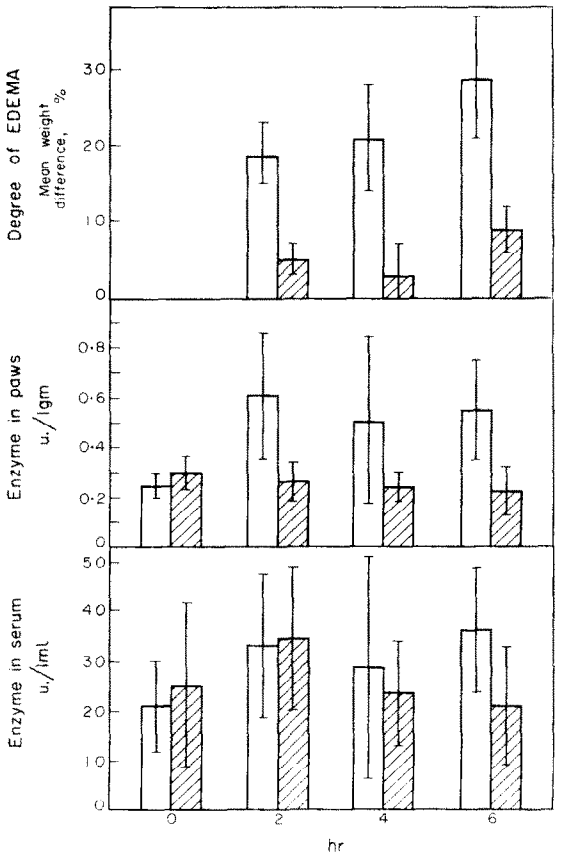


FIG. 1. Development of edema and changes in the ATEE-esterase activity in paws (wet weight) and in serum, in terms of units (1 unit corresponds to 1 $\mu$M of substrate hydrolysed in 1 min at 37°). Open columns: without inhibitor, shaded columns: inhibitor administered. Standard errors of the mean indicated.

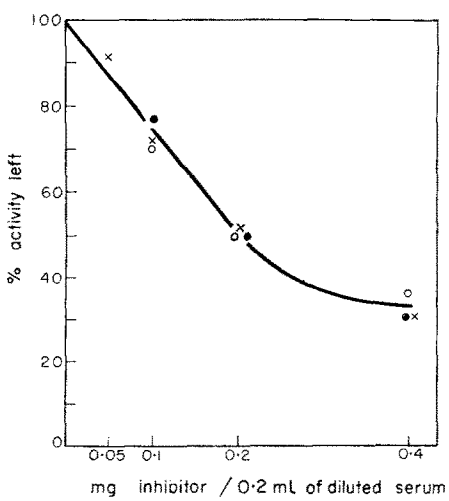


FIG. 2. Inhibition of ATEE-esterase activity in serum by the inhibitor from potatoes. × ○ ● represent three independent determinations. Incubation mixture: 0·2 ml of 30 times diluted rat serum, 0·1 ml of the inhibitor solution, 0·3 ml of the veronal buffer pH 8·0, 0·1 ml of phenol red 0·02% and 0·5 ml 0·02 M ATEE. Incubated at 37°, 30 min, read at 520 $\mu$m.

the degree of inflammation, as measured by the weight increase of paws, and was lowered after the administration of IPP. A relatively high activity in serum showed an insignificant tendency to increase in untreated rats and to decrease in later phases after the administration of IPP. Moreover, an uncomplete inhibition of the activity of extracts from inflamed tissues and from normal serum by IPP was observed *in vitro* (Fig. 2). There was always a good correlation between this inhibition and the anti-inflammatory activity of different samples of IPP. Some synthetic drugs showed also anti-esterase activity (phenylbutazone, procain). Consequently, an enzyme is supposed to exist in the inflamed tissue and in serum, which bears some resemblance to chymotrypsin in its hydrolytic activity on ATEE, but which differs in its susceptibility to inhibition by the peptidic type of IPP and also by its thermolability (activity in serum disappears at 55° within 1 min). Meanwhile, a permeability-increasing activity was found previously by other authors in the homologous serum of the rat.[5] Now, it was possible to find, in DEAE-cellulose fractions of serum proteins, a correlation between the distribution of the esterolytic activity on ATEE and the ability to produce inflammatory reactions after the subplantar injection to the rat. In this experiment, serum proteins from DEAE-cellulose columns were eluted stepwise with buffer systems (A—0·05 M sodium phosphate pH 8·0, B—0·05 M sodium phosphate pH 7·5 + 0·02 M sodium chloride, D—0·05 M sodium phosphate pH 7·0 + 0·04 M sodium chloride, E—0·05 M sodium phosphate pH 6·0 + 0·08 M sodium chloride, F—0·05 M sodium chloride + 1 M HCl pH 2·0). The dialysed and lyophilised fractions were reconstituted to the original volume of

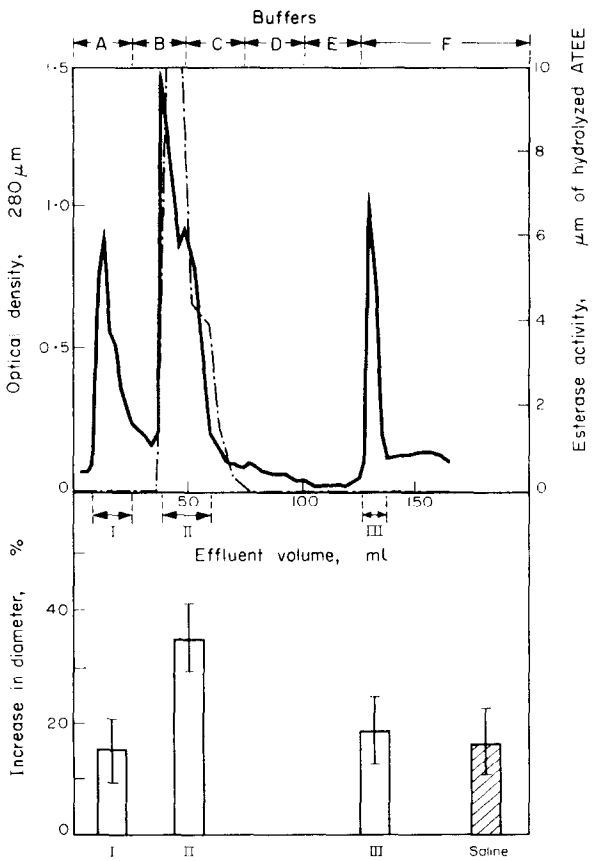


FIG. 3. Upper part: Chromatographic separation of rat serum proteins on DEAE-cellulose columns. Stepwise elution with buffers. Protein distribution ($D_{280}$) is outlined by the solid line, hydrolytic activity on ATEE of 0·5 ml effluent by the broken line. In the lower part: Mean increases in the dorsoplantar diameter of rat paws 15 min after the injection. Effects of saline injections for comparison represented by a shaded column. Standard errors of the mean are indicated.

serum with saline and injected into paws of rats in the volume of 0·1 ml. The degree of inflammation was expressed as the difference in mm between the original dorsoplantar diameter of the paw and values attained 15 min after the injection of the tested solution (Fig. 3). The protein fraction containing most of the esterase activity produced also maximum inflammatory reactions.

The hypothetical enzyme plays possibly a significant role in the process of inflammation and experiments are planned to elucidate this role.

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## Biosynthesis of norepinephrine and norsynephrine in the perfused rabbit heart

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CONSIDERABLE evidence has been obtained that confirms the postulated pathway by Blaschko[1] for the stepwise formation of norepinephrine. The decarboxylation of dopa and the β-hydroxylation of dopamine to form norepinephrine has recently been studied extensively *in vivo* and *in vitro*. Virtually nothing is known about the formation of dopa from tyrosine in mammalian enzyme systems, except that this reaction occurs in adrenals[2] and in certain sympathetic nerve tissues.[3] It remains, therefore, to be elucidated whether organs innervated by sympathetic nerves such as the heart are able to perform all the enzymatic reaction steps from tyrosine leading to norepinephrine.

In recent studies on β-hydroxylation of dopamine, it was shown that after infusion of rabbits with dopamine-$^{14}$C, high amounts of norepinephrine-$^{14}$C were found in the hearts.[4] The norepinephrine in the heart may have accumulated through uptake from the blood or through synthesis in the heart itself. The ability of the heart to form norepinephrine from various possible precursors was tested in the present study. The rabbit heart was perfused with dopamine-$^{14}$C, tyramine-$^{14}$C, and tyrosine-$^{14}$C, and the formation of catechols and β-hydroxylated compounds was investigated.

Rabbits were pretreated with 100 mg iproniazid/kg and killed 16 hr later. The thorax was immediately opened and the heart removed and perfused by the Langendorff technique with oxygenated (95% $O_2$, 5% $CO_2$) Krebs–Ringer bicarbonate solution at 38°. A stopcock on the cannula allowed continuous infusion of radioactive compounds into the perfusing fluid. The outflow from the hearts was collected during the perfusion time. The radioactive compounds in the heart and perfusate were isolated and analyzed by a method that involves extraction into organic solvents, ion exchange, alumina and paper chromatography, and acetylation procedures.[5, 6]

The radioactivities in the β-hydroxylated products isolated from heart tissue after perfusion with the various precursors are shown in Table 1. It is evident that 1 to 3 per cent of the administered dopamine-$^{14}$C was converted to norepinephrine which was accumulated in the heart tissue. In the perfusate, unchanged dopamine-$^{14}$C and two O-methylated metabolites, 3-methoxytyramine and normetanephrine, were found (Table 2). No norepinephrine was detected in the perfusate.

After perfusion with tyramine-$^{14}$C, norsynephrine was isolated from the heart tissue (Table 1). Only negligible amounts of unchanged tyramine-$^{14}$C were found. The perfusate contained tyramine-$^{14}$C and other metabolites which have not been identified at the present time (Table 2). Neither dopamine-$^{14}$C nor norepinephrine-$^{14}$C has been detected in the heart tissue or in the perfusate after perfusion with tyramine-$^{14}$C.