

## ALI-ESTERASE INHIBITORS AND GROWTH

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The family of enzymes with which we are concerned in this paper were formerly known as esterases and often confused with lipases. However, since they are incapable of splitting ordinary fats and act preferentially on simple aliphatic esters such as tributyrin, methyl butyrate, and triacetin, they have more recently been renamed ali-esterases (Richter and Croft, 1942). Unlike the cholinesterases, they do not hydrolyse esters of choline. Furthermore, they can be distinguished from the cholinesterases by their insensitivity to inhibition by eserine and prostigmine (Easson and Stedman, 1937; Mendel and Rudney, 1943).

Ali-esterases have been found in higher and in lower forms of life—in animal tissues, micro-organisms, and plants; there is probably no cell which does not contain this type of enzyme. Nevertheless, the physiological functions and the natural substrates of the ali-esterases are still unknown.

The first selective inhibitor of ali-esterases was found in the course of investigations concerned with the function of the pseudo-cholinesterase (Mendel and Rudney, 1943). It was noticed that tri-*o*-cresyl phosphate (TOCP), a selective inhibitor of the pseudo-cholinesterase in rats (Mendel and Rudney, 1944), is an even more potent inhibitor of ali-esterases in this species (Mendel and Mortimer, 1944; cf. Mendel and Myers, 1953). The ali-esterases (tributyrynases) of serum, liver, and many other tissues can be inhibited *in vivo* almost completely without noticeable ill effects, and such ali-esterase inhibition over a period of many weeks does not affect the well-being or growth of young rats. This discovery raised the question whether, in the course of evolution, the ali-esterases might have lost much of their importance for the animal kingdom, while retaining their essential role in the metabolism of lower forms of life—of viruses, micro-organisms, fungi, plants, and malignant cells. Should this prove true, then ali-esterase inhibition

might be a means of interfering selectively with the metabolism and possibly with the growth of invading micro-organisms and other parasitic cells.

A beginning was made in 1944 to test this hypothesis with TOCP on tubercle bacilli (Mendel and Fraser, 1944), which contain large amounts of ali-esterase (Mendel and Hawkins, 1944). The experiments at that time were only a partial success, because the inhibitory action of TOCP, according to our recent investigations, seems to be too slow to keep pace with the formation of new esterase in rapidly multiplying cells. Nevertheless, the idea continued to exert its fascination and has now led to experiments in which it has been found possible, with more potent ali-esterase inhibitors, to arrest the growth of germinating seeds and tubercle bacilli and to inhibit completely the growth of malignant cells without affecting the growth of normal cells in the same tissue culture.

### *Ali-esterase Inhibitors*

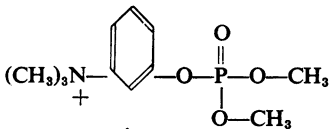
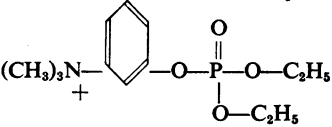
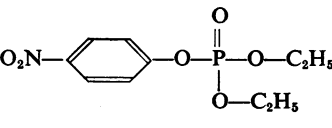
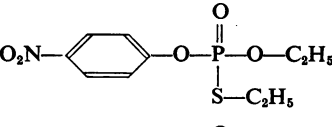
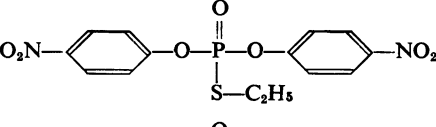
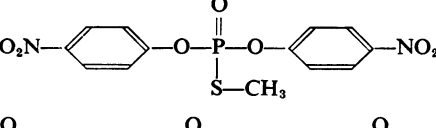
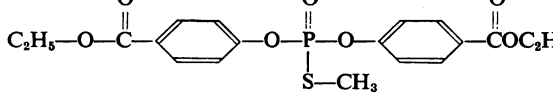
The ali-esterases of sera have recently been divided into two groups by Aldridge (1953a). The results of our experiments support this classification, which might be extended to include similar enzymes found in tissues. The A-type ali-esterase is not inhibited by phosphate tri-esters (Aldridge, 1953a; cf. Myers and Mendel, 1949; Mendel and Myers, 1953), presumably because these esterases are capable of hydrolysing phosphate esters without being inhibited in the process (Aldridge, 1953b). The B-type ali-esterases are inhibited by very low concentrations of tri-*o*-cresyl phosphate, di-isopropyl fluorophosphonate (DFP), and diethyl *p*-nitrophenyl phosphate (E600, paraxon) (Mendel and Mortimer, 1944; Mendel and Myers, 1953; Myers, 1952; Aldridge, 1953a). To these two types of ali-esterase we might add a third, which can only be inhibited by comparatively high concentrations of the phosphate tri-esters mentioned above. An esterase of this kind, which we

might designate as the C-type, is found in mammalian brain (Mendel and Myers, 1953).

The ali-esterases found in lower forms of life often resemble these mammalian ali-esterases in their susceptibility to inhibitors and in their sub-

strate specificity. Yeast cells, for example, contain an A-type esterase, and a B-type ali-esterase has been found in a hepatoma of the mouse; the ali-esterases in mycobacteria, sarcoma, carcinoma, and lymphosarcoma, and the related esterases in

TABLE I  
INHIBITION OF THE ALI-ESTERASE (TRIBUTYRINASE) AND TRUE CHOLINESTERASE ACTIVITIES OF RAT BRAIN HOMOGENATE BY ORGANIC PHOSPHATE ESTERS

Compound No. and Designation	Structure	Molar Concentration Causing 50% Inhibition		Ratio of 50% Inhibitory Concentrations: Ali-E/True ChE
		Ali-esterase	True Cholin-esterase	
(1) 3-0412 ..		$4 \times 10^{-2}$	$8 \times 10^{-9}$	$5 \times 10^6$
(2) 3-0340 ..		$5 \times 10^{-3}$	$1 \times 10^{-7}$	$5 \times 10^4$
(3) E600 ..		$1.4 \times 10^{-7}$	$1.4 \times 10^{-8}$	10
(4)		$4 \times 10^{-7}$	$3 \times 10^{-7}$	1.3
(5)		$1.6 \times 10^{-6}$	$4 \times 10^{-6}$	0.4
(6)		$8 \times 10^{-7}$	$1 \times 10^{-5}$	0.08
(7) E 1907 ..		$1 \times 10^{-6}$	$1 \times 10^{-5}$	0.10

The esterase activities were measured manometrically by the Warburg technique at 37° C. and pH 7.4, using 0.03 M acetyl-*D*-methylcholine as a specific substrate for the true cholinesterase (Mendel, Mundell, and Rudney, 1943) and 0.2% tributyrin as substrate for the ali-esterase. The phosphate esters were incubated with a 2% suspension of rat brain for 30 min. before the substrate was added.

Compounds 1 and 2 were synthesized by Roche Products, Ltd., England; compounds 3, 4, 5, and 6 by Professor J. A. A. Ketelaar and Mr. H. R. Gersmann, of the University of Amsterdam, Holland; and compound 7 by Dr. G. Schrader, Elberfeld, Germany.

seeds, resemble more the C-type ali-esterase of mammalian brain. Although TOCP is very suitable as a selective inhibitor of the B-type ali-esterase of serum and liver, intestinal mucosa, kidney, and testis *in vivo* (Mendel and Mortimer, 1944; Mendel and Myers, 1953; Myers and Simons, 1953), it has proved inadequate in the present investigation because the esterases of bacteria, seeds, and tumour cells cannot be inhibited rapidly and effectively enough by TOCP. One of the first problems was, therefore, to find for the C-type ali-esterase a more potent inhibitor, which would not at the same time inhibit the true cholinesterase.

The ali-esterase (tributyryl-esterase) of rat brain homogenate was used as a model of the C-type esterase in these experiments; this has the advantage that the inhibitory action of various compounds on the true cholinesterase and on the ali-esterase can readily be determined with the same tissue preparation under identical experimental conditions. It had been observed that DFP is capable of inhibiting various types of esterase (Webb, 1948), and we found that DFP also inhibits the ali-esterase of brain in a concentration of  $10^{-5}$  M. To determine the structural properties which might be expected to characterize a selective inhibitor of ali-esterases, we studied a series of organic phosphate esters, the most interesting of which are shown in Table I. Within this series, the ratio of inhibitory activities towards ali-esterase and true cholinesterase ranges from 5 million to 0.1; it appears that the selectivity of these inhibitors is largely determined by the same factors which determine the selectivity of reversible inhibitors such as prostigmine and the selectivity of esterase activity towards various substrates (Myers, 1953a, 1953b).

The greatest inhibitory effect on the ali-esterase was observed with E600 (No. 3); this compound still inhibits the cholinesterase somewhat better than it inhibits the ali-esterase, and consequently it is not suitable for experiments on ali-esterase inhibition *in vivo*. Further alterations in the structure of the phosphate molecule have led to compounds (Nos. 6 and 7) which inhibit the ali-esterase of brain in one-tenth of the concentrations which inhibit the true cholinesterase. These compounds are not stable in aqueous solution, and the inhibition *in vivo* after a single injection lasts only for a few hours (Myers, Mendel, Gersmann, and Ketelaar, 1952). Before we can attempt to inhibit the growth of pathogenic cells in an animal, we shall need a more selective ali-esterase inhibitor which gives a longer-lasting

inhibition. However, we know now that it is possible to alter the selectivity of an inhibitor over a very wide range, and we can expect to obtain a greater selectivity towards ali-esterase by following up the results given in Table I.

Most of the experiments on the growth of bacterial and malignant cells *in vivo* have been carried out with E600 as inhibitor of the ali-esterase activity. Not only is E600 the most potent of these inhibitors against the C-type ali-esterase, but it is also one of the most stable compounds of this kind. In contrast to the cells of normal mammalian tissues, the malignant and the bacterial cells investigated contain either insignificant amounts of cholinesterase or none at all; moreover, it would not be expected that any cholinesterase present would be concerned with processes of cell growth. Thus the inhibitory action of E600 on cholinesterase need not be taken into account in experiments on cell growth *in vitro*.

#### Esterase Inhibitors and the Growth of Germinating Seeds

The esterases of seeds resemble the ali-esterases of mammalian tissues in their ability to hydrolyse simple aliphatic esters and in their insensitivity to eserine, but may differ in other respects (Jansen, Jang, and MacDonell, 1947). However, since the acetylcholinesterase of wheat can be selectively inhibited by organic phosphates (Jansen, Nutting, and Balls, 1948), it was of interest to determine whether the esterase inhibitors would have any effect on the germination and growth

TABLE II  
EFFECT OF ESTERASE INHIBITORS ON THE GERMINATION AND GROWTH OF OAT SEEDS

Inhibitor	Conc. (mg.%)	Effect on Growth of Seeds			Esterase Activity <i>in vitro</i> (% of Control)
		% Which Germinated	% Which Grew	Average Weight of Shoot Plus Root (mg. per Seed)	
E600 ..	0	79	75	103	100
	1	71	62	71	84
	5	73	38	19	49
	10	56	25	8	32
	25	0	0	0	12
E605 ..	0	88	80	102	100
	10	76	50	26	48
	25	72	29	7	19
DFP ..	0	81	75	97	100
	1	90	80	95	98
	5	80	67	75	95
	10	73	59	50	74
	25	69	22	14	52

The effect of the esterase inhibitors on the growth of the seeds was usually determined after 6 days' incubation; all values given are calculated on the basis of the total number of seeds used in each experiment.

Esterase activity was measured manometrically by the Warburg technique at 25° C. and pH 7.4 with 0.2% tributyrin as substrate; a 2% homogenate of oat seeds was used.

of seeds. In these experiments, wheat (*Triticum vulgare*) and oat (*Avena sativa*) seeds were first soaked for 24 hours in tap water with and without the inhibitor; they were then placed on filter paper, moistened daily with fresh solutions of the same kind, and allowed to germinate in a dark

room for three days at about 13° C. and then at 20° C.

The effects of E600, a thiophosphate analogue (E605), and DFP on the growth of oat seeds and on the esterase activity are summarized in Table II. It can be seen that the growth of the germin-

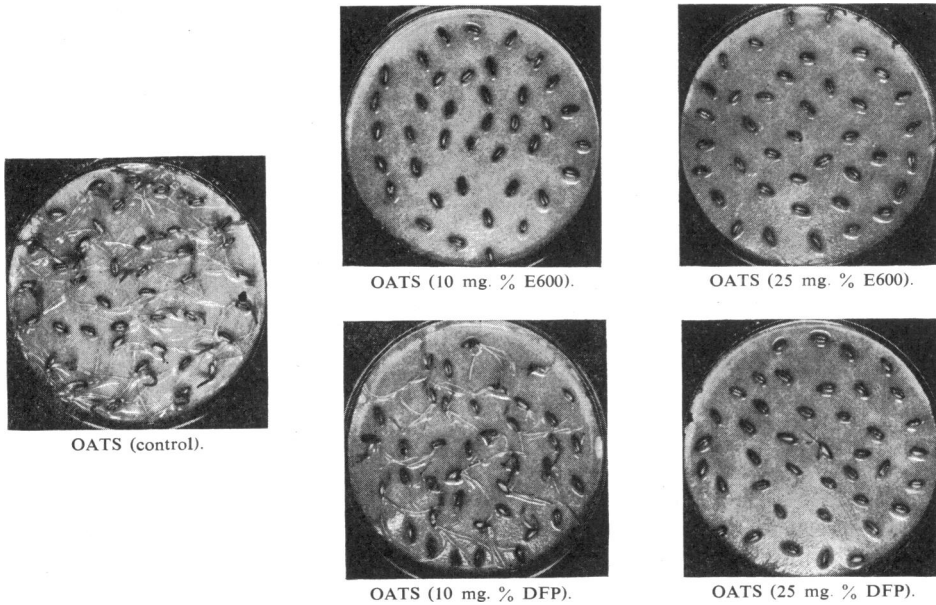


FIG. 1.—Effect of E600 and DFP on the germination of oat seeds after 6 days.

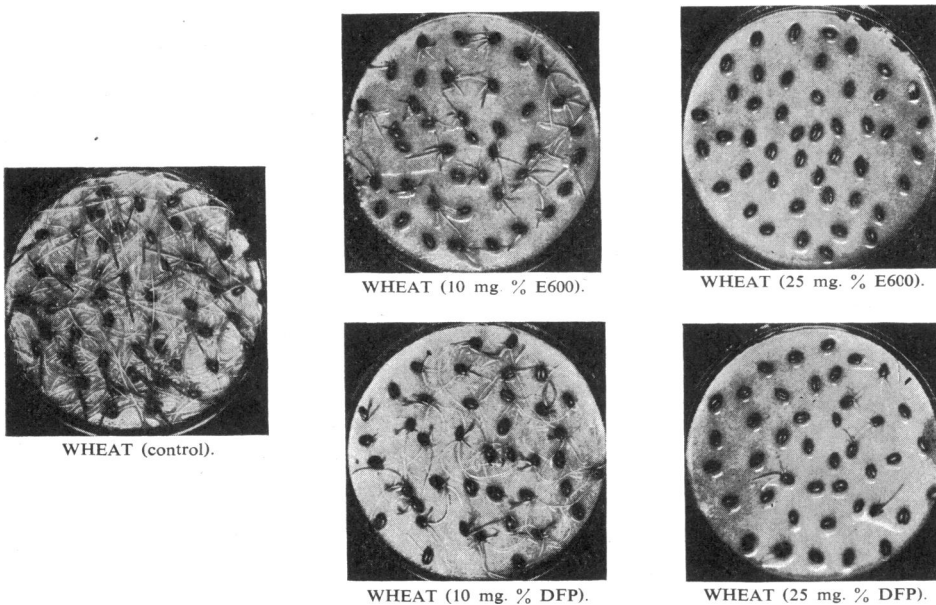


FIG. 2.—Effect of E600 and DFP on the germination of wheat seeds after 6 days.

ating seeds is delayed by all three phosphate esters in concentrations which partially inhibit the esterase activity and that the germination can be completely prevented with a concentration of inhibitor sufficient to inhibit the esterase activity by about 90%. The inhibition of the esterases by E600 is readily reversible, and it was found that the inhibition of growth is also abolished when the inhibitor solution is replaced by tap water.

A marked difference between the esterases of wheat and oat seeds was observed. The esterase of wheat hydrolyses triacetin and nitrophenyl acetate, but has little activity towards tributyrin (cf. Jansen, Nutting, and Balls, 1948); this acetyl-esterase is about as sensitive to inhibition by DFP as it is to inhibition by E600. The esterase of oats, on the other hand, hydrolyses tributyrin most rapidly, has little activity towards triacetin, and is more sensitive to E600 than to DFP. A correlation with these results was seen in the effects of these inhibitors on the germination and growth of the seeds. Germination and growth of oat seeds are considerably more sensitive to E600 than to DFP (Fig. 1); the shoots of the wheat seeds were about equally sensitive to both inhibitors, while the roots were usually more sensitive to E600 (Fig. 2). These results all substantiate the hypothesis that the inhibition of growth of the germinating seeds by organic phosphate esters is due to inhibition of the esterase activity.

#### *Ali-esterase Inhibitors and Growth of Mycobacterium Tuberculosis*

Most of these experiments were carried out with the strain *M. tuberculosis t. humanus* H37RV grown at 36–37° C. in a Dubos medium containing albumin from human plasma. After inoculation with a small amount of a well-grown culture, growth of the bacilli produced a turbidity of the medium within 3–4 days. All experiments were done in duplicate and the results were practically always identical.

The growth of the tubercle bacilli was inhibited by E600 in concentrations as low as 0.05 mg. %

(0.5 µg./ml.; ca.  $2 \times 10^{-6}$  M) (Table III). The inhibition of growth was reversible, indicating that the inhibitor was not acting as a non-specific protoplasmic poison. The higher the initial concentration of E600 the longer was the period during which growth was prevented; in some experiments with 1 mg. % E600 the bacilli only started to grow after a period of six weeks. However, once growth had reached a visible level, it continued at the same rate as had been observed in the controls, regardless of how long it had been inhibited previously. The cessation of the inhibitory effect after various periods of time is probably due to the breakdown of E600 and the reappearance of ali-esterase activity. It is known that E600 decomposes slowly in aqueous solution; according to the data of Aldridge and Davison (1952), the original concentration would decrease to one-tenth in about 40 days in a phosphate buffer of pH 7.6 at 37° C. However, E600 breaks down much more rapidly in the Dubos medium than in a phosphate buffer of the same pH; it appeared that its hydrolysis was catalysed by the albumin in the Dubos medium (cf. Aldridge, 1953b) so that the concentration of E600 decreased to one-tenth in 5–7 days.

Why the inhibition of growth considerably outlasts the presence of the inhibitor in the medium has not yet been fully explained. In any case, the prolonged effect of E600 is not due to breakdown products of the inhibitor which, as we have ascertained, do not influence the growth of tubercle bacilli when present in the same molar concentration as E600; nor did we observe an effect with tri-p-nitrophenyl phosphate—a compound which is much more labile than E600 but a very poor esterase inhibitor.

A few experiments were done with other strains of *M. tuberculosis t. humanus*, one of which was resistant to 100 U. of streptomycin. All of these strains were equally or slightly more sensitive to the action of E600 in the Dubos medium. An experiment was also done according to the method of Price; it was found that the growth of tubercle

TABLE III  
EFFECT OF ESTERASE INHIBITORS ON CULTURES OF TUBERCLE BACILLI (H37RV) IN MEDIUM OF DUBOS AT 37° C

Days after Inoculation	E600 (mg.%)						Days after Inoculation	DFP (mg.%)			
	0	0.01	0.05	0.1	0.3	1		0	1	5	20
3	+	+	—	—	—	—	4	+	—	—	—
4	+	+	—	—	—	—	5	+	—	—	—
5	++	++	+	—	—	—	6	+++	+	—	—
6	++++	++++	+++	+	—	—	8	++++	+++	+	—
8	++++	++++	++++	++++	++	—	12	++++	++++	++++	—*
12	++++	++++	++++	++++	++++	—					
16	++++	++++	++++	++++	++++	++					

\* Subculture in Dubos medium without DFP ++ after 22 days.

TABLE IV  
EFFECT OF E600 ON CULTURES OF TUBERCLE BACILLI IN THE MEDIUM OF DUBOS AT DIFFERENT TEMPERATURES

Days after Inoculation	<i>M. tuberc. t. humanus</i>						<i>M. avium</i>		
	36° C. E600 (mg.%)			38.5° C. E600 (mg.%)			38.5° C. E600 (mg.%)		
	0	0.05	0.1	0	0.05	0.1	0	0.1	1.0
5	+	-	-	+	-	-	+++	++	+
6	++	-	-	+++	-	-	++++	++++	+
7	+++	+	-	++++	+	-	++++	++++	++
9	++++	+	-	++++	+++	++	++++	++++	
13	++++	++	+	++++	++++	++++			

bacilli in sputum smears on glass slides in haemolysed human blood was strongly inhibited for several days by 1 mg. % E600.

The effects of E600 on the growth of tubercle bacilli were not altered significantly by variations in the pH of the medium between 6 and 7.5, nor did the age of the culture and the number of bacilli used for inoculation influence the results appreciably. However, E600 had a stronger inhibitory effect on the growth at 36° C. than at 38.5° C. Since tubercle bacilli multiply somewhat faster at 38.5° than at 36° C., a more rapid inhibition of the ali-esterase, and thus a higher concentration of E600, should be required at 38.5° C. in order to keep pace with the rate of esterase synthesis at the higher temperature. This suggestion is supported by the finding that higher concentrations of E600 were necessary to inhibit the more rapid growth of avian tubercle bacilli (Table IV), and that the even faster growth of saprophytic mycobacteria at 37° was not affected by 1 mg. % E600, while their slow growth at 22° could be partially inhibited by this concentration.

There are some facts which cannot be readily explained as yet: The inhibition of growth by E600 was potentiated by the addition of 2% glycerol to the Dubos medium, and E600 was considerably less active when Tween 80 was omitted from the medium; these results may be due to a change in the permeability of the bacilli to the phosphate ester.

TABLE V  
INHIBITION OF ESTERASE ACTIVITY OF TUBERCLE BACILLI BY E600 AND DFP AT 37° C.

Conc. E600 (mg.%)	pH		Conc. DFP (mg.%)	pH	
	Initial	After 30 min.		Initial	After 60 min.
0	7.0-7.1	6.2	0	7.0-7.1	6.0
0.01	7.0-7.1	6.4	0.05	7.0-7.1	6.1
0.03	7.0-7.1	6.6	0.2	7.0-7.1	6.2
0.1	7.0-7.1	6.8	1.0	7.0-7.1	6.9
0.3	7.0-7.1	7.0	5.0	7.0-7.1	7.0

E600 or DFP was added to 2.5 mg. of tubercle bacilli suspended in a volume of 5 ml. After a subsequent addition of tributyrin in a final concentration of 0.1%, the esterase activity was estimated from the decrease in pH caused by the hydrolysis of the substrate.

Experiments on the ali-esterase activity of the tubercle bacilli showed that the hydrolysis of triacetin and tributyrin is almost completely inhibited by 0.3 mg. % E600 and partially inhibited by 0.01-0.1 mg. % after 30 min. incubation (Table V). Thus there is a close correlation between the concentrations of E600 which inhibit the ali-esterase activity and the concentrations which prevent the growth of the tubercle bacilli. Analogous results were obtained with DFP; this compound only inhibits the ali-esterase activity of tubercle bacilli in a concentration of about 1 mg. % (Table V), and the same concentration was necessary to exert a bacteriostatic effect (Table III).

#### *An Ali-esterase Inhibitor (E600) and Growth of a Transplantable Mouse Lymphosarcoma in Tissue Culture*

As it is essential to study the action of the substance to be tested on both malignant cells and their normal prototypes, most experiments were carried out with cultures of lymph-glands of a healthy mouse and with four different strains of cells all derived from the lymphosarcoma MB (T86157) described by de Bruyn, Korteweg, and Kits van Waveren (1949), de Bruyn 1949, 1950), and de Bruyn and Gey (1952). A brief characterization of the cell strains follows:

Strain I contains lymphoblasts and fibroblasts. The lymphoblasts produce a tumour, whereas the fibroblasts do not.

Strain II contains only fibroblasts which do not produce a tumour.

Strain III originated in one out of several tubes in which the fibroblasts died out, and apparently a change occurred in the lymphoblasts. Early inoculations showed that the altered cells could produce tumours. However, after growing in tissue culture for approximately four and a half months they lost their ability to produce a tumour in originally susceptible mice.

Strain IV is a mixed strain, containing the fibroblasts of strain II and the altered lymphoblasts of strain III.

The strains derived from the lymphosarcoma were maintained in continuous culture for more than five years. The normal lymphoid tissue was grown for

one or two weeks or directly taken from a lymph-gland of a healthy mouse.

The method employed in these experiments was the roller-tube method of Gey. The medium consisted of various proportions of Tyrode's solution, mouse-embryo extract, human-cord serum, and chicken plasma. E600 was used in a concentration of 10 mg. %, which was found to inhibit the ali-esterase of this particular lymphosarcoma almost completely.

**Results.**—Thus far six series of experiments were carried out. In all of these experiments E600 inhibited the growth of the lymphoblasts of strain I (Fig. 3), whereas no effect could be seen on the altered, non-malignant lymphoblasts of strains III and IV (Fig. 4) and on the lymphoid cells of cultures of normal lymph-glands. Moreover, E600 had no effect on the fibroblasts of strains I, II, and IV, and on the fibroblasts of cultures of normal lymph-glands. Thus only the malignant cells, i.e., the lymphoblasts of strain I, were affected.

It was noticed that E600 breaks down fairly rapidly in the tissue culture medium. However,

no difference could be observed between the growth of the cells in the control medium and the growth in a medium containing a breakdown product of E600 (p-nitrophenol) in the same molar concentration as E600. It is very likely, therefore, that the inhibitory effect of E600 on growth is due to the inhibition of the ali-esterases present in the malignant cells.

#### DISCUSSION

While our investigations on the ali-esterases were in progress, Smith, Worrel, and Swanson (1949) reported that chloramphenicol inhibits the esterase activity of *E. coli*. We were not able to confirm this finding; a different mechanism for the bacteriostatic action of chloramphenicol has meanwhile been suggested by Gale and Paine (1951). We have also tested various other chemotherapeutic agents, e.g., isonicotinic acid hydrazide and nitrofurvaldehyde semicarbazone, against the ali-esterase of mammalian tissues and of tubercle

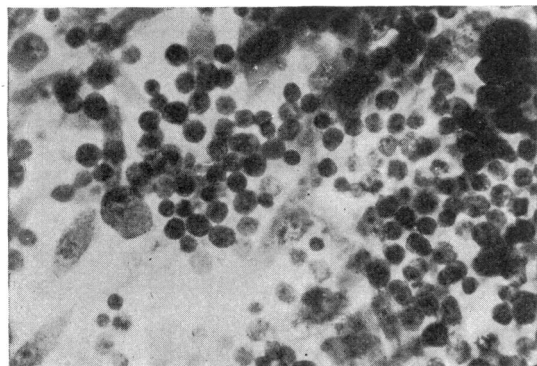


FIG. 3 A

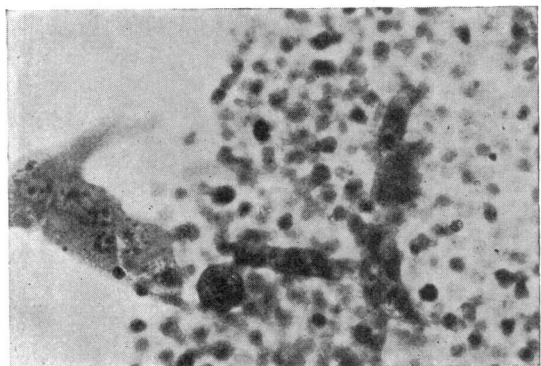


FIG. 3 B

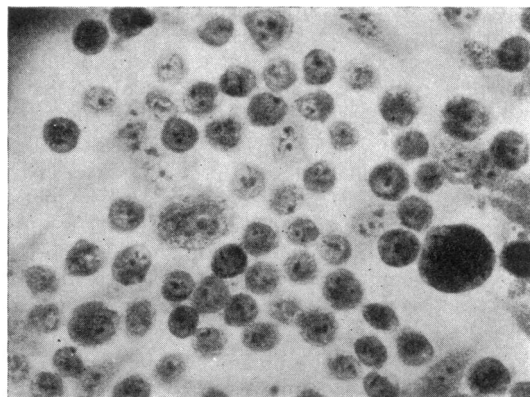


FIG. 4 A

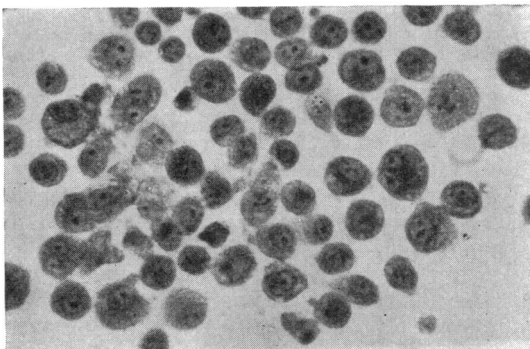


FIG. 4 B

FIG. 3.—(A) Fixed and stained cultures of the malignant Strain I in control medium. Fibroblasts and lymphoblasts in good condition; mitoses. (B) Fixed and stained cultures of Strain I in the medium containing E600. Fibroblasts in good condition; lymphoblasts in very poor condition.

FIG. 4.—(A) Fixed and stained cultures of the non-malignant Strain IV in control medium. Lymphoblasts in good condition; mitoses. (B) Fixed and stained cultures of Strain IV in medium containing E600. Lymphoblasts in good condition; mitoses.

bacilli; in no case did we observe any significant esterase inhibition by bacteriostatic concentrations. However, our experiments with organic phosphate esters on germinating seeds, tubercle bacilli, and malignant cells appear to support the hypothesis that growth of lower forms of life can be arrested by inhibition of their ali-esterases. We are, of course, aware that our results might be due to inhibition of other hydrolytic enzymes by the phosphate tri-esters (Jansen, Nutting, Jang, and Balls, 1949; Hartley and Kilby, 1952). Nevertheless, we consistently find a correlation between the inhibition of ali-esterase activity and the inhibition of growth, and the hypothesis has provided a fruitful basis for our investigations.

#### SUMMARY

The finding, that an animal is not adversely affected by prolonged inhibition of ali-esterases, led to the hypothesis that the ali-esterases might be enzymes which, in the course of evolution, have lost much of their significance for the animal kingdom, while retaining their essential role in the metabolism of lower forms of life. In order to test this hypothesis the ali-esterases of seeds, tubercle bacilli, and malignant cells have been inhibited by a number of organic phosphate esters. The results of these experiments are as follows:

1. The germination and growth of seeds are inhibited by ali-esterase inhibitors such as diethyl p-nitrophenyl phosphate (E600), diethyl p-nitrophenyl thiophosphate (E605) and di-isopropyl fluorophosphonate (DFP). A close relationship exists between the degree of ali-esterase inhibition and the degree of growth inhibition.

2. The growth of human tubercle bacilli in a Dubos medium is inhibited by E600 in concentrations as low as 0.5  $\mu\text{g./ml.}$  and by DFP in a concentration of 10  $\mu\text{g./ml.}$  Again there is a close correlation between inhibition of ali-esterase activity and inhibition of growth.

3. In tissue cultures the growth of the malignant cells (lymphoblasts) of a mouse lymphosarcoma is inhibited by E600 in a concentration which inhibits the ali-esterase activity. The non-malignant fibroblasts in the same lymphosarcoma, the lymphoid cells and fibroblasts of normal lymph glands, and a non-malignant variation of the lymphoblasts originating from the mouse lymphosarcoma all grow normally in the presence of the concentration of E600 by which the growth of the malignant cells is inhibited.

These results may provide a basis for a new departure towards a rational chemotherapy of tuberculosis and neoplastic disease.

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