presence of urine it was usually necessary to separate the layers by centrifugation. The combined extracts were then evaporated to dryness *in vacuo* at room temperature, and to the residue, 10 ml. of 20 per cent sodium thiosulphate solution, and one drop of methyl orange were added. The solution was then titrated with 0·1N HCl until the indicator remained red for 10–15 sec. (V_1). 30 min. later two drops of phenolphthalein were added, and the solution titrated to a pink colour with 0·1N NaOH (V_2). A blank estimation was made by treating 10 ml. of thiosulphate in a similar way (B_1 and B_2). Then $(V_1 - V_2) - (B_1 - B_2)$ = volume of 0·1N HCl equivalent to the NaOH liberated by the reaction. Since each molecule of Thiotepa (mol. wt. 189·3) contains 3 ethylene imine rings, multiplication of this volume by 18·93/3 gives the number of mg. of Thiotepa in the sample taken.

As pure crystalline Thiotepa was not available it has not been possible to check every aspect of the analytical procedure which must therefore form the basis for further study. However, estimations of the drug in standard ampoules were consistent with the stated content and several analyses of a solution kept at −15° over a week gave reproducible results.

I am greatly indebted to Lederle Laboratories through the courtesy of Dr. J. R. Wilson for unpublished information on which this method was based. I also wish to acknowledge the help of Dr. B. E. Tomlinson of the Department of Pathology, The General Hospital, Newcastle upon Tyne, in whose department these studies were made.

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The Action of Aryloxyaliphatic Acids on the Permeability of Blood Vessels

SIR,—It has been shown recently that certain aryloxyaliphatic acids have anti-inflammatory properties (Northover and Subramanian, 1961) and it was of interest, therefore, to study the correlation of chemical structure and biological activity in this series.

The method for measuring the effect of drugs on the permeability of peritoneal blood vessels has been described in detail elsewhere (Northover, 1962). Briefly, the method consists of following the movements of azovan blue dye from the circulation into the peritoneal fluid of mice given 4 ml. of 0·9 per cent saline solution intraperitoneally. The concentration of dye in the peritoneal fluid at the end of 1 hr. is a measure of the permeability of the peritoneal blood vessels to plasma albumin. Male mice weighing between 25 and 30 g. were arranged in groups of 12 animals each, and the groups were treated with graded doses of the substances under test. 0·1 ml. of a neutralised solution or suspension of the substance under investigation was administered subcutaneously to each animal. 30 min. later, 4 ml. of 0·9 per cent saline solution at 38° were given intraperitoneally to each mouse. Immediately afterwards each animal was

given 0·2 ml. of a 0·5 per cent solution of azovan blue in 0·9 per saline through a lateral tail vein. The peritoneal fluid was withdrawn 1 hr. after it had been administered, centrifuged, and its optical extinction measured.

The optical extinction for the group is expressed as a per cent of the value obtained with the untreated control group and this is termed the per cent permeability for the group. The test is performed with various doses of the substance under investigation, hence it is possible to calculate the dose which would produce a per cent permeability of 50 per cent, and this is termed the ED50 dose.

TABLE I

PERMEABILITY-INHIBITING ACTION OF ARYLOXYALIPHATIC ACIDS

Name	ED50 dose mg./kg.	Per cent permeability with 200 mg./kg.
Phenoxyacetic acid	—	70
p-Acetylphenoxyacetic acid	—	62
o-Aldehydophenoxyacetic acid	Inactive*	—
p-Carboxyphenoxyacetic acid	—	58
Resorcinoxydiacetic acid	Inactive	—
Vanilloxyacetic acid	Inactive	—
o-Chlorophenoxyacetic acid	42	35
p-Chlorophenoxyacetic acid	173	44
o-Cresoloxyacetic acid	—	62
p-Cresoloxyacetic acid	158	41
Eugenoxyacetic acid	—	72
Thymoxyacetic acid	40	13
4-n-Hexylresorcinoxyacetic acid	78	36
α-Naphthoxyacetic acid	37	13
β-Naphthoxyacetic acid	104	43
α-(*p*-t-Butylphenoxy) propionic acid	50	22
2-(*p*-(1,1-Dimethylpropyl)phenoxybutyric acid	51	27

* Indicates that the per cent permeability was not significantly different ($P > 0.05$) from the control All quoted figures are significantly different ($P < 0.05$) from the controls.

A number of aryloxyaliphatic acids were tested in this way and the results are given in Table I. Phenoxyacetic acid itself has little activity, whereas some of its substituted derivatives are active. Phenolic, aldehydic, acyl, and carboxylic substituent groups produce little activity. The greatest activity seems to be associated with alkyl and aryl substituent groups, although chlorine substitution also produces some activity. It appears that by increasing the size of the alkyl substituent group the activity is increased.

None of the compounds tested was acutely toxic to mice in doses up to 200 mg./kg. which was the highest dose tested. With one exception, all the compounds tested were either available commercially or were synthesised in the laboratory and the melting points checked against those in the literature. For the oxyacetic acid of n-hexylresorcinol we find no record in the literature of its preparation and properties so its identity is only provisionally established. Its equivalent weight as an acid was 252, and on analysis it gave 14 per cent carbon, 9 per cent hydrogen and 17 per cent oxygen.

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