Table 4. *Estimation of ascorbic acid in normal blood plasma (recovery experiments)*

Method	Ascorbic acid, mg. /100 ml.			Error of recovery (%)
	In normal plasma	Amount added	Total found	
Spectrophotometric	-0.79	0.5	1.28	-3.4
	-1.0	0.4	1.41	+2.5
	-1.0	1.0	1.96	-4.0
	-0.82	0.7	1.53	+1.4
	-0.82	1.2	2.18	-1.7
Photoelectric	-0.76	0.5	1.24	-4.0
	-1.03	0.4	1.43	+1.2
	-1.03	1.0	2.0	-3.0
	-0.84	0.7	1.56	+2.8
	-0.84	1.2	2.01	-2.5
Roe & Kuether (1943)	-0.72	0.5	1.2	-4.0
	-1.0	0.4	1.4	0.0
	-1.0	1.0	1.96	-4.0
	-0.88	0.7	1.6	+2.8
	-0.88	1.2	2.04	-3.3

ments (Table 4) known amounts of ascorbic acid were added to plasma which was treated in exactly the same way as normal plasma.

DISCUSSION

The advantages of the method described here are rapidity and simplicity. The reagent used is inexpensive and can easily be prepared and no special care need be taken regarding temperature or time of reaction. The possible interfering substances are reductones, reductic acid and cysteine. In Roe & Kuether's method hexoses, pentoses and glucuronic acid may interfere. Both methods seem to give about the same accuracy of result but the colorimetric method using *perinaphthindanetrione* is simpler and quicker.

SUMMARY

1. *perinaphthindanetrione* hydrate is used as a new reagent for the spectrographic estimation of ascorbic acid in pure solutions, blood plasma and urine.
2. The 2-nitro*perinaphthindanetrione* hydrate can also be used to estimate ascorbic acid in pure solutions.
3. This reagent is easily prepared, inexpensive, specific and stable.
4. The new method when compared with that of Roe & Kuether (1943) proved to be simpler and takes less time.

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The Inhibition of Histaminase by Amidines

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In a study of the inhibitory action of certain amidine derivatives on the enzyme amine oxidase it was found that the inhibition depended not only on the presence of the amidine group but on the configuration of the molecule as a whole (Blaschko & Duthie, 1945*a*). This study has recently been extended to a

number of isothioureas derivatives (Fastier & Hawkins, 1951).

Histaminase ('diamine oxidase') is also inhibited by some of these amidines (Blaschko & Duthie, 1944), and in the present paper we have examined the inhibitory action in more detail. The results show

that the relation between chemical structure and inhibitory action is quite different for the two enzymes which catalyse the oxidative deamination of amines.

METHODS

The enzyme was obtained from pig kidney; its preparation from acetone-dried powder has already been described (Blaschko & Duthie, 1945*b*). By dialysing the extracts for a few hours against tap water the O_2 consumption of the control can be reduced without affecting the activity of the enzyme, and this is not altered in the presence of the amidines. The latter were added, together with the substrate (cadaverine), from the side arm of the manometer vessel at the start of the experiment. The flasks were set up as follows: main flask: 1.6 ml. extract; side bulb: 0.2 ml. of 5×10^{-2} -M-cadaverine dihydrochloride and 0.2 ml. water or inhibitor; inner tube: 0.3 ml. N-KOH. The gas phase was O_2 and the temperature 37.5°.

The amidines examined include those studied by Blaschko & Duthie (1945*a*) and some of the *isothiourea* derivatives tested by Fastier & Hawkins. Decamethylene diguanidine dihydrochloride—prepared from the commercially available carbonate—was tested as an inhibitor of both amine oxidase and histaminase.

The inhibitors were examined in concentrations of 10^{-3} M or less. Percentage inhibitions were calculated from the amounts of O_2 consumed during the first 30 min. of the reaction.

RESULTS

Straight-chain monoamidines:

$$\text{CH}_3 \cdot (\text{CH}_2)_n \cdot \text{C}(\text{:NH}) \cdot \text{NH}_2 \text{ (Table 1).}$$

The inhibitory action of the members of this group was small. Table 1 shows that there was no marked effect of chain length.

Straight-chain diamidines:

$$\text{NH}_2(\text{NH}:)\text{C} \cdot (\text{CH}_2)_n \cdot \text{C}(\cdot:\text{NH}) \cdot \text{NH}_2 \text{ (Table 2).}$$

The compounds of this series were more active as inhibitors than the monoamidines. The length of the polymethylene chain was without influence on the degree of inhibition; substances with a chain length from $n=7$ to $n=16$ had approximately the same inhibitory action. This is in marked con-

trast to the results with amine oxidase (Blaschko & Duthie, 1945*a*), where the inhibitory action is at a maximum for the compound with eleven CH₃ groups.

Table 1. Inhibition of diamine oxidase by straight-chain monoamidines

(Type compound $\text{CH}_3(\text{CH}_2)_n\text{C}(:\text{NH})\text{NH}_2$. Inhibitor concentration 10^{-8}M .)

<i>n</i>	...	...	...	3	4	8	10	14
Inhibition (%)				14	6	24	16	3
				26		33		

Diamidines:

The inhibitory activity of the substances in this group was greater, and they were therefore examined in a molar concentration of 10^{-4} . The compound with one CH_2 group was most, and that with ten CH_2 groups least active as inhibitor, but the substances with two, three, four, five and six CH_2 groups all had very much the same inhibitory activity. This group includes propamidine ($n=3$) and pentamidine ($n=5$), both strong inhibitors of amine oxidase.

The closely related compound phenamidine,

also was a strong inhibitor of diamine oxidase, 10^{-4} M inhibiting by 90 %.

Guanidine derivatives. The two monoguanidines,

and

were too insoluble to be tested in a concentration of 10^{-3}M ; in 10^{-4}M concentration the inhibitions were 4 and 5 % respectively; this is probably within the limits of experimental error.

The series of straight-chain diguanidines,

included substances with a high affinity for diamine oxidase, and the effect of chain length on inhibitory

Table 2. *Inhibition of diamine oxidase by straight-chain diamidines*

(Type compound $\text{NH}_2(\text{NH})\text{C}(\text{CH}_3)_n\text{C}(\text{:NH})\text{NH}_2$. Inhibitor concentration 10^{-3}M .)

<i>n</i>	...	...	7	8	9	10	11	12	12	14	16
Inhibition (%)			80	73	77	92	74	57	76	76	78
						68		78	79	82	85
						84					

Table 3. *Inhibition of diamine oxidase by certain aromatic diamidines*

(Type compound $\text{NH}_2(\text{NH}_2\text{C}-\text{C}_6\text{H}_4-\text{O}(\text{CH}_2)_n-\text{C}_6\text{H}_4-\text{C}(\text{NH}_2)\text{NH}_2$. Inhibitor concentration 10^{-4}M .)

<i>n</i>	...	...	1	2	3	4	5	6	10
Inhibition (%)			81	70	49	61	73	63	25
			94	74	51	59	72	61	31
			95				63 75	72	

action was marked. Fig. 1 gives the percentage inhibitions observed with a concentration of 10^{-4}M , plotted against chain length. With the lower members of the series inhibitions were high, although slightly irregular, but beyond $n=8$ inhibitions fell off, and for the compound with $n=18$ inhibitory action was slight.

The relation between chain length and inhibition in this series was characteristically different from that found for amine oxidase. Fig. 1 also shows the percentage inhibitions observed with amine oxidase for an inhibitor concentration of 10^{-3}M ; most of these data are from an earlier paper (Blaschko & Duthie, 1945*a*), but recent results with decamethylene diguanidine have been included.

Fig. 1. Inhibition of amine oxidases by diguanidines. ●, histaminase, inhibitor concentration 10^{-4}M ; ○, amine oxidase, inhibitor concentration 10^{-3}M . Each dot or circle represents the mean of several experiments.

A diguanidine derivative of spermine,

gave an inhibition of 88% in 10^{-3}M concentration.

Monoisothioureia derivatives. Ten members of the series $\text{CH}_3(\text{CH}_2)_n\text{S}\cdot\text{C}(\text{:NH})\text{NH}_2$ have been tested, the first nine ($n=0-8$) and the twelfth ($n=11$). In a concentration of 10^{-4}M , only the first, *S*-methyl isothioureia, caused an inhibition of 25%; on raising the concentration to 10^{-3}M , values of 52 and 69% were obtained. None of the higher homologues affected the enzyme in a concentration of 10^{-4}M .

The first three members of the series:

viz. *S*-benzyl, *S*-2-phenylethyl and *S*-3-phenyl-*n*-propyl isothioureia, were without appreciable inhibitory activity at 10^{-4}M concentration.

Diisothioureia derivatives.

Eight compounds of this series were examined, the first six ($n=1-6$), the decamethylene and the dodecamethylene derivatives. Fig. 2 gives a summary of our experiments in which these substances were tested in a molar concentration of 10^{-4} . The methy-

lene derivative ($n=1$) was found to be without inhibitory action, and *SS'*-ethylene diisothioureia ($n=2$) was less active than the higher members which all inhibited the enzyme to about the same extent.

Fig. 2. Inhibition of histaminase by diisothioureias. Inhibitor concentration 10^{-4}M . Each dot represents one experiment.

Stilbamidine and dimethylstilbamidine (Table 4). Stilbamidine is a very strong inhibitor of diamine oxidase, and Table 4 shows that the dimethyl

Table 4. Inhibition of diamine oxidase by stilbamidine and dimethylstilbamidine

Inhibitor concentration (M) ...	10^{-3}	10^{-4}	10^{-5}
Inhibition by stilbamidine (%)	100	89	50
Inhibition by dimethylstilbamidine (%)	100	87	61

derivative has very much the same affinity; even in 10^{-5}M concentration the inhibitions were still about 50%. The percentage inhibitions differ slightly from those previously reported (Blaschko & Duthie, 1944); this is possibly due to differences in experimental conditions, since the earlier results were obtained with a fresh aqueous extract of pig kidney.

Aneurin. According to Zeller (1939), aneurin is an inhibitor of diamine oxidase. This was confirmed in our experiments, but the percentage inhibitions were rather low. With 10^{-3}M -aneurin the inhibition was 40% and with 10^{-4}M -aneurin 5%.

DISCUSSION

Guanidine was the first amidine compound which was found to inhibit histaminase (Blaschko, 1938). Zeller (1938) showed that the two diguanidines, arcaine ($n=4$) and Synthalin A ($n=10$) were even stronger inhibitors. The experiments reported in this paper show that it is always in the series of compounds with two amidino groups that the strong inhibitors are found; the monoamidines are less active. This also finds a parallel in the observations of King, Lourie & Yorke (1938) on the trypanocidal

action of amidine derivatives *in vitro*, the diamidines being more active than the monoamidines.

Many of the diamidines studied in this paper cause an increase in the blood histamine concentration (MacIntosh & Paton, 1949), due to the release of histamine from the tissues. Some of the compounds which were particularly active as inhibitors of histaminase in our experiments were also highly active in MacIntosh & Paton's experiments. There is no general parallelism between antihistaminase activity and histamine release, but in view of the observations here reported the possibility must be considered that some of these substances exert an antihistaminase effect also in the living animal.

SUMMARY

1. The inhibitory action of amidine derivatives on the histaminase of pig kidney has been studied.
2. Diamidines, diguanidines and diisothiurea derivatives have an inhibitory action.
3. Stilbamidine and dimethylstilbamidine are strong inhibitors of the enzyme.
4. The relation between chain length and inhibition has been studied and the results are discussed.

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A New Type of Preparative Electrophoresis Cell

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The electrophoresis apparatus devised by Tiselius (1937) is now available in many laboratories. It is predominantly used for analytical work, but can also be used for preparing electrophoretically pure materials in small quantities by virtue of the fact that after the migration of the various components of a mixture has taken place for a time, the fastest and slowest may be isolated by lateral displacement of the cell sections, the boundaries having been compensated to suitable positions. The disadvantages of this method are twofold. In the first place, at the conclusion of one such separation the whole apparatus must be taken out of the refrigerated bath, and at least partially dismantled in order that the desired material may be removed, and if more material is required, the apparatus must be re-assembled, refilled, and replaced in the bath before another run can be started. In the second place, when removing components obtained in this way, it is difficult to avoid dilution with the buffer which fills the remainder of the cell compartment. These difficulties have to a certain extent been overcome by a semi-continuous procedure developed by MacLagan & Bunn (1947) with the insertion of capillary tubes inside the channel of the long centre

section Tiselius U-tube, but the method is not entirely satisfactory to operate.

EXPERIMENTAL

Description of cell. The type of cell described here enables both the disadvantages mentioned to be overcome, while still using the standard auxiliary equipment belonging to the analytical cell. In principle it is based on the type of cell first described by Svensson (1942) and later simplified and modified by him (Svensson, 1946). Fig. 1 shows that the cell is identical with the standard Tiselius model with the long centre section, except for the fact that five capillary openings are provided, two at the top of either arm of the cell, two at the bottom, and one in the base section which joins the two arms. These openings allow material to be introduced into and withdrawn from the cell, while the apparatus as a whole remains in the refrigerated bath. In order that this should be possible, arms of capillary tubing are attached to the outlets either by rubber sleeves or by ground-glass joints, and by means of taps in these tubes the flow of liquid into and out of the cell may be controlled. The formation of protein-buffer boundaries in this cell takes place by flow of liquid, the