THE INHIBITION OF TISSUE RESPIRATION AND ALCOHOLIC FERMENTATION AT DIFFERENT CATABOLIC LEVELS BY ETHYL CARBAMATE (URETHAN) AND ARSENITE

by

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In an earlier communication[1] an explanation of the action of urethan (and arsenite) on malignant growth has been given, which was based upon two assumptions:

(1) the respiration of tumours *in situ* is speeded up to the maximal turnover number of the respiratory enzyme system by the high energy requirement of the processes leading to the synthesis of new cell material, while the respiration of the other tissues *in situ* proceeds at the "normal" rate, which is much below the maximal capacity of their enzyme system;

(2) a certain amount of urethan (or arsenite) always blocks the same portion of the inhibitor-sensitive enzyme(s).

From these assumptions it follows that the respiration of a tumour will be affected by a low concentration of urethan (or arsenite), which will not yet have a measurable effect on the respiration of normal tissue. In the latter case the turnover number of the residual part of the urethan-(arsenite-)sensitive enzyme(s) can be sufficiently increased to maintain the original level of respiration. So we consider the catabolic energy producing processes to be the limiting factor in malignant growth, while normal growth or cell production requires much less energy than the catabolic system(s) of the tissue might produce. Normal tissues can therefore compensate the effect of small concentrations of inhibitors of catabolism by what might be called their reserve capacity, while in tumour tissue, where all capacity is in use, catabolism will be considerably inhibited by the same concentrations of inhibitors. Hence malignant growth will be inhibited or completely checked by a certain urethan (arsenite) concentration, while the other tissues of the patient treated with urethan (arsenite) will continue to synthesize cell material and to multiply at their original, much lower, rate.

One model experiment, the synthesis of aneurin pyrophosphate from small and large amounts of aneurin added to blood, which corroborated this theory, has already been published[1]. We have now studied the influence of urethan and arsenite on the respiration of animal tissues and the fermentation of bakers' yeast, the rate of these processes being adjusted to various levels by different means.

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EXPERIMENTAL PART

Kidney and liver

Kidney and liver of albino rats weighing about 200 g were minced by means of Cooper scissors and suspended in a Ringer solution (medium II as described by KREBS[2]). The oxygen consumption was measured during 60 minutes at 37° C in Warburg respirometers (about 100 mg fresh tissue per vessel of about 15 ml volume; total volume of suspension in main compartment 1.8 ml; 0.1 ml 5% KOH in centre well; 0.2 ml inhibitor solution (in Ringer) or Ringer solution in side arm).

In a first series of experiments we compared the influence of urethan and arsenite on the O_2 uptake in air, in oxygen and in oxygen in the presence of 2,4-dinitrophenol (DNP) in the medium (final concentration $1.6 \cdot 10^{-4}$ *m*). In a second series we carried out similar experiments in oxygen/nitrogen mixtures of 20.4, 38.6, 51.8, 67.0, 80.3, and 99.6% oxygen. All results are expressed in

$$Q_{O_2} = \frac{\mu l\ O_2}{\text{mg initial dry weight} \times \text{hours}}.$$

Yeast

The influence of urethan and arsenite on the fermentation of bakers' yeast was studied anaerobically at 27° C in Warburg vessels of the same dimensions as mentioned above (3.3 mg fresh yeast in 1.7 ml 0.1 *m* phosphate-acetate buffer of p_H 5.6, containing 2.5% glucose and 0.1% ammonium sulphate, to which various amounts of urethan (or arsenite) could be added; nitrogen atmosphere). The carbon dioxide production was measured for periods of 20 minutes during several hours. All measurements were carried out in duplicate.

To obtain good duplicate measurements it was necessary to lift the manometer from the thermostat and shake the contents of the vessels vigorously by knocking the wooden support of the manometer several times against the table. After this manipulation the respirometers were reinserted in the bath. 4 minutes later—the time required to re-establish temperature equilibrium—the manometers were read.

While the CO_2 production remains practically constant in the medium from which ammonium salts have been omitted, it gradually increases to a maximum in glucose-ammonium sulphate solution, probably as a consequence of the synthesis of protein from the ammonium ions[3], without noticeable increase in the number of cells.

The number of cells was counted at the beginning and at the end of the experiment in a BÜRGER-TÜRK counting chamber after 40-fold dilution without staining. The cell suspension was introduced into the chamber by the method described for blood cells by SMITS AND FLORIJN[4].

Sodium arsenite solutions were always prepared by dissolving As_2O_3 p.a. in an excess of NaOH and neutralizing afterwards.

Urethan was determined by the procedure of SCHAFFER, LEBARON AND WALKER[5]. This determination could even be carried out in solutions containing large amounts of ethanol, if this alcohol was first removed completely by 15 minutes' distillation at p_H 7, whereby — as control experiments showed — no urethan was decomposed.

RESULTS

Kidney and liver

Table I gives the results of the experiments in air, oxygen and oxygen in the presence of DNP.

Fig. 1 exhibits a more detailed description of the results of one of those experiments. In the lower part of the figure the oxygen consumption curves for one hour are given. Each point represents the oxygen uptake for a period of fifteen minutes. As these curves show that the O_2 uptake decreases only very little during one hour and as they have the same slope, we have plotted in the upper part of the figure the percentual inhibition by urethan, calculated for the whole period of one hour, against the concentration of urethan in the medium. Similar types of curves would have been obtained if the inhibition had been calculated for any period of 15 minutes.

The contents of Table I as well as the data represented by Fig. 1 show that tissue minces metabolize at the highest rate in oxygen in the presence of DNP, and at the

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TABLE I

INFLUENCE OF URETHAN AND ARSENITE ON THE OXYGEN UPTAKE OF KIDNEY AND LIVER MINCES AT DIFFERENT CATABOLIC LEVELS

100 mg fresh tissue in 2.0 ml (total volume) of KREBS' medium II; I in air; II in oxygen; III in oxygen with $1.6 \cdot 10^{-4}$ *m* DNP in the medium. The values given are the mean values of the experiments; the range of these values is stated between brackets.

Tissue	Molar concentr. of inhibitor		I				II				III			
			Numb. of exp.	Qo_2		Aver. inhib. in pCt	Numb. of exp.	Qo_2		Aver. inhib. in pCt	Numb. of exp.	Qo_2		Aver. inhib. in pCt
	Ureth.	Ars.		Control	Inhibitor added			Control	Inhibitor added			Control	Inhibitor added	
kidney	0.028		2	— 9.2 (9.0–9.4)	— 9.75 (9.65–9.85)	—6	2	— 18.55 (18.0–19.1)	— 19.35 (18.6–20.1)	—4	2	— 26.3 (25.8–26.8)	— 24.4 (24.2–24.6)	+7
kidney	0.056		2	— 9.2 (9.0–9.4)	— 9.45 (9.3–9.6)	—3	3	— 18.0 (17.0–18.5)	— 17.6 (16.5–19.0)	+2	3	— 25.7 (24.8–26.3)	— 23.2 (22.6–23.6)	+10
kidney	0.084		2	— 9.2 (9.0–9.4)	— 9.35 (9.25–9.45)	—2	4	— 17.45 (16.3–18.5)	— 16.1 (15.4–17.3)	+8	3	— 26.7 (25.8–27.2)	— 21.8 (20.9–23.4)	+16
kidney	0.112		2	— 9.2 (9.0–9.4)	— 8.7 (8.55–8.85)	+6	2	— 18.55 (18.0–19.1)	— 16.6 (16.3–16.9)	+11	2	— 26.3 (25.8–26.8)	— 20.7 (20.0–21.4)	+21
kidney	0.140		4	— 8.3 (7.75–9.1)	— 7.7 (7.1–8.65)	+7	4	— 17.7 (16.3–18.5)	— 15.0 (13.9–16.0)	+15	4	— 23.9 (21.6–24.8)	— 18.85 (17.9–19.4)	+21
kidney	0.168		2	— 9.2 (9.0–9.4)	— 8.3 (8.25–8.35)	+10	5	— 17.85 (15.9–18.7)	— 13.9 (12.8–15.8)	+22	2	— 26.3 (25.8–26.8)	— 17.9 (16.9–18.9)	+32
kidney	0.336		3	— 8.3 (6.6–9.4)	— 3.55 (3.3–3.8)	+57	14	— 17.45 (15.6–18.7)	— 7.0 (6.25–9.0)	+60	6	— 25.2 (22.4–26.4)	— 6.0 (5.3–6.4)	+76
liver	0.112		2	— 5.9 (5.75–6.05)	— 5.45 (5.3–5.6)	+7	2	— 8.2 (8.0–8.4)	— 7.2 (6.5–7.9)	+13	2	— 9.6 (9.3–9.9)	— 7.9 (7.7–8.1)	+18
liver	0.168		2	— 5.9 (5.75–6.05)	— 4.9 (4.85–4.95)	+17	2	— 7.4 (6.6–8.2)	— 6.45 (5.8–7.1)	+13	2	— 9.6 (9.3–9.9)	— 7.55 (7.4–7.7)	+21
liver	0.336		2	— 5.9 (5.75–6.05)	— 3.9 (3.8–4.0)	+34	4	— 7.5 (6.65–8.15)	— 4.8 (4.15–5.1)	+36	2	— 9.6 (9.3–9.9)	— 4.75 (4.5–5.0)	+51
kidney		$5 \cdot 10^{-5}$	4	— 10.8 (9.6–12.2)	— 10.45 (9.5–11.8)	+3	4	— 22.8 (20.4–24.6)	— 20.1 (19.5–20.8)	+12	4	— 29.8 (27.2–32.8)	— 22.4 (20.4–23.4)	+25
kidney		$25 \cdot 10^{-5}$	4	— 10.35 (9.0–11.1)	— 8.3 (6.8–9.25)	+20	4	— 23.45 (22.0–27.2)	— 13.0 (11.8–14.5)	+45	4	— 28.8 (27.6–32.4)	— 11.2 (10.9–11.7)	+62

lowest in air, while the rate is intermediate in oxygen in the absence of DNP. In general the percentual inhibition by urethan increases proportionally to the urethan concentration. In air and in oxygen in the absence of DNP, however, a slight activation of the O_2 consumption by small concentrations of urethan is observed. This activation is more pronounced in air (lowest level of metabolism) than in oxygen (intermediate level).

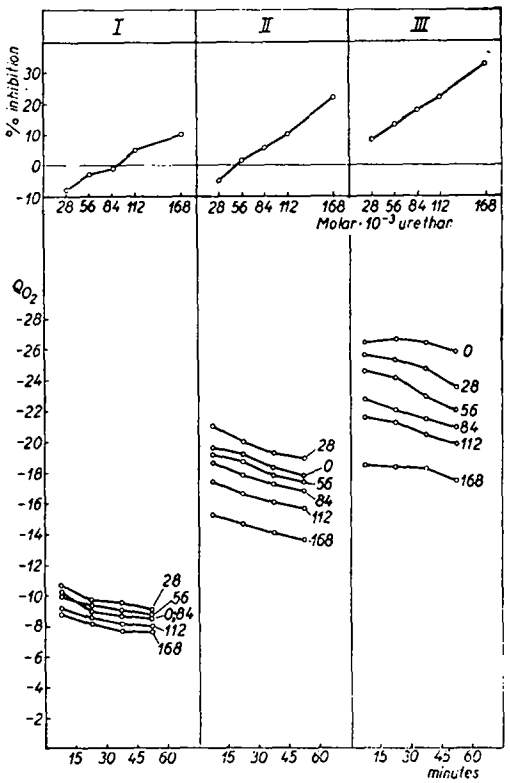

Fig. 1. Experiment 52. Below: Q_{O_2} of kidney mince in the presence of different concentrations of urethan, plotted against time in minutes. The figures to the right of the curves indicate the urethan concentrations, expressed in 10^{-3} moles per l. I in air, II in oxygen, III in oxygen and DNP (in the medium). Above: Percentual inhibition plotted against urethan concentrations

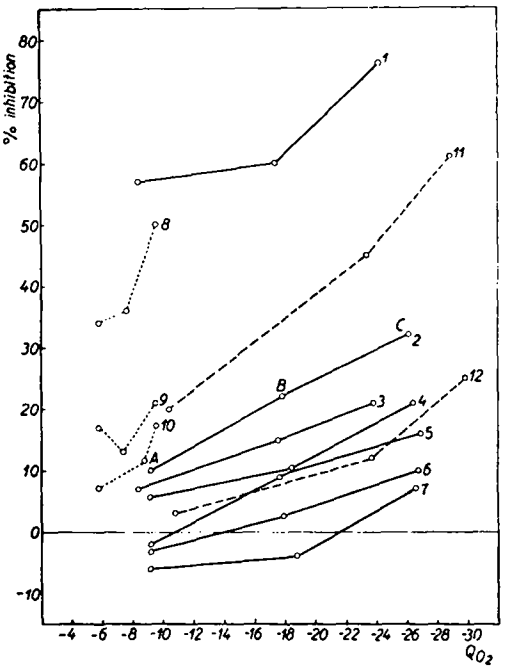

Fig. 2. Percentual inhibition plotted against Q_{O_2} for different concentrations of urethan and arsenite

o———o kidney mince: 1. 0.336 *m* urethan; 2. 0.168 *m* urethan; 3. 0.140 *m* urethan; 4. 0.112 *m* urethan; 5. 0.084 *m* urethan; 6. 0.056 *m* urethan; 7. 0.028 *m* urethan

o-------o liver mince: 8. 0.336 *m* urethan; 9. 0.168 *m* urethan; 10. 0.112 *m* urethan

o– – – –o kidney mince: 11. $25 \cdot 10^{-5}$ *m* arsenite; 12. $5 \cdot 10^{-5}$ *m* arsenite

Similar activations were always observed at low levels of metabolism either with urethan or with arsenite. When the oxygen uptake is decreased below that in air by using oxygen/nitrogen mixtures with still lower oxygen content this activation is increased up to 22 pCt.

In Fig. 2 the percentual inhibition by a given concentration of urethan or arsenite is plotted against the Q_{O_2} of the non-inhibited tissue sample. For example, curve 2 refers to an experiment on kidney mince in air (point A), oxygen (point B) and oxygen and DNP (point C) in the presence of 0.168 *m* urethan. With one exception the percentual

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inhibition increases with $-Q_{O_2}$. In this figure the activation by small concentrations of urethan at low catabolic levels can again be observed.

The results of the experiments in gas mixtures of various oxygen contents are collected in Table II. The data of this table have been used for drawing Fig. 3, in which the percentual inhibition is plotted against Q_{O_2}. The general trend of these curves is similar to those of Figure 2: the percentual inhibition by a given concentration of urethan or arsenite increases with $-Q_{O_2}$ of the non-inhibited tissue sample.

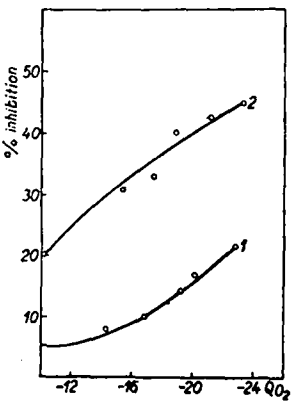

Fig. 3. Percentual inhibition plotted against Q_{O_2}. Kidney mince. Different partial oxygen pressures in gas phase. 1. 0.140 *m* urethan; 2. $25 \cdot 10^{-5}$ *m* arsenite

TABLE II

INFLUENCE OF URETHAN AND ARSENITE ON THE OXYGEN UPTAKE OF KIDNEY MINCES AT DIFFERENT CATABOLIC LEVELS

100 mg fresh tissue in 2.0 ml (total volume) of KREBS' medium II; different oxygen content of gas-phase. The values given are the means of 4 experiments; the range of these values is stated between brackets.

pCt of O_2 in O_2/N_2 mixtures	Inhibitor 0.140 *m* urethan			Inhibitor $25 \cdot 10^{-5}$ *m* arsenite		
	Q_{O_2}		Aver. inhib. in pCt	Q_{O_2}		Aver. inhib. in pCt
	Control	Inhibitor added		Control	Inhibitor added	
20.4	— 10.1 (9.05–10.8)	— 9.6 (8.65–10.1)	+5	— 10.35 (9.0–11.1)	— 8.3 (6.8–9.25)	+20
38.6	— 14.4 (13.5–15.9)	— 13.25 (12.4–14.3)	+8	— 15.35 (14.8–15.9)	— 10.55 (9.0–11.6)	+31
51.8	— 16.8 (15.5–17.5)	— 15.1 (13.9–15.9)	+10	— 17.3 (15.9–18.5)	— 11.6 (10.8–13.35)	+33
67.0	— 19.25 (17.1–20.8)	— 16.5 (14.7–18.1)	+14	— 19.8 (18.6–22.4)	— 11.8 (11.45–12.9)	+40
80.3	— 20.2 (18.9–21.6)	— 16.9 (15.5–18.7)	+17	— 21.2 (20.0–24.7)	— 12.2 (11.25–13.5)	+42
99.6	— 22.8 (22.0–24.8)	— 17.9 (17.4–19.4)	+22	— 23.45 (22.0–27.2)	— 13.0 (11.8–14.5)	+45

Yeast

Curves 0 and 4 of Fig. 4, in which the rate of CO_2 production (μl in 20 minutes) is plotted against time, gives the general shape of the curves representing the fermentation rate in a glucose solution with and without ammonium sulphate. As in the former case the fermentation rate increases without any significant increase in the number of cells (see legend to Fig. 4) the influence of a given concentration of inhibitor on the fermentation rate at different rates of fermentation can be studied in one Warburg respirometer.

Fig. 4 reproduces the results of a number of experiments on the inhibition of fermen-

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tation by urethan carried out simultaneously. Fig. 5 shows the results of similar experiments regarding the action of arsenite. The increase of the percentual inhibition by a given concentration of inhibitor with increasing fermentation rate is quite obvious. The decrease of percentual inhibition at later stages of fermentation, at which the fermentation rate is still slowly increasing, can be explained by the observed slow decomposition of urethan. In the experiment reproduced in Fig. 4 the urethan concentration was diminished by 15–20 % 400 minutes after the beginning of the experiment.

A decrease of the inhibition by arsenite in the last stages of fermentation was also observed. This phenomenon is more difficult to explain as it is not likely that arsenite will disappear from the yeast suspension under the prevailing anaerobic conditions. It might be possible that it is withdrawn from the enzymes by linkage to the proteins or other nitrogeneous substances formed during fermentation in the presence of ammonium sulphate.

Activation of fermentation, analogous to the activation of oxygen

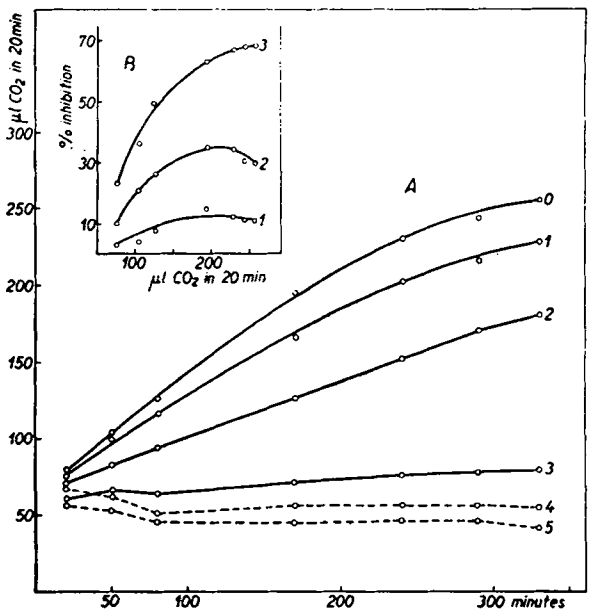

Fig. 4. (A) CO_2 production of yeast with different urethan concentrations

o———o ammonium sulphate added: 0. no urethan added; 1. 0.020 *m* urethan; 2. 0.099 *m* urethan; 3. 0.198 urethan

o– – – –o without ammonium sulphate: 4. no urethan added; 5. 0.198 *m* urethan

Number of cells per µl: at the beginning of the experiment $4.6 \cdot 10^4$; at the end of the experiment of curve o: $4.9 \cdot 10^4$; at the end of the experiment of curve 4: $4.4 \cdot 10^4$

(B) Percentual inhibition plotted against rate of fermentation. Urethan concentrations as in A

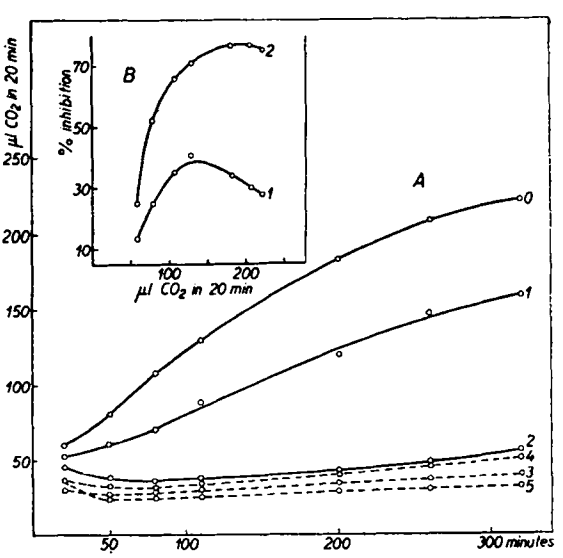

Fig. 5. (A) CO_2 production of yeast with different arsenite concentrations

o———o ammonium sulphate added: 0. no arsenite added; 1. $6 \cdot 10^{-5}$ *m* arsenite; 2. $15 \cdot 10^{-5}$ *m* arsenite

o– – – –o without ammonium sulphate: 3. no arsenite added; 4. $6 \cdot 10^{-5}$ *m* arsenite; 5. $15 \cdot 10^{-5}$ *m* arsenite

(B) Percentual inhibition plotted against the rate of fermentation. Arsenite concentrations as in A

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uptake in kidney and liver minces, at low rates of metabolism by low concentrations of inhibitor was also observed, as can be seen from Fig. 5.

DISCUSSION

All experimental results seem to confirm our original hypothesis that a given concentration of urethan or arsenite blocks a definite portion of the sensitive enzyme(s). In consequence the relative inhibition by a given concentration of inhibitor will depend upon the turnover number of the enzyme(s) and will generally increase with increasing catabolic level. Our experiments can, however, be criticized from various angles.

a. Regarding our first experiment, in which different rates of oxygen uptake were obtained by working in air, oxygen or oxygen with addition of DNP to the suspension the question could be raised whether, in the presence of DNP, the same enzymes are concerned as in the absence of this substance. We believe this to be true in view of work published by others on the action of DNP[6, 7]. Moreover, the assumption of two different catabolic pathways, one of these working only or mainly in the presence of DNP, does not seem likely. As all the enzymes of the system fit together so adequately, replacement of one or more of them with simultaneous increase of activity does not appear probable.

This possible objection does not apply to our second experiment (different mixtures of nitrogen and oxygen in the gas phase).

b. Objections may be raised against comparing yeast containing varying amounts of protein or at least varying amounts of nitrogeneous substances. As these substances are formed by the yeast in a medium containing glucose as only organic matter, it is rather improbable that the enzyme system present in each yeast cell would be increased. But even if the same enzymes would be formed as those already present, the increase of percentual inhibition with increasing catabolic rate would become still more outspoken, if reduced to the same enzyme concentration.

In the medium used in our experiments an increase of the number of yeast cells does not occur. If, however, a mixture of different factors like wort is added, the number of cells increases to threefold in 4 hours. We will postpone the discussion of the form of the fermentation rate/time curve to a later date.

The phenomenon of increasing inhibition by the same concentration of inhibitor with increasing metabolic activity, the latter depending upon the substrate concentrations at the surfaces of the various enzymes, can be derived for a single isolated enzyme from the theory of STRAUS AND GOLDSTEIN[8]. In this theory an extension and generalization of the theory of MICHAELIS AND MENTEN is given, based upon the consideration that the concentration of the enzyme as compared to the substrate concentration cannot be considered to be negligible in all cases. To derive the necessary equations the following symbols are used:-

E enzyme
S substrate
E_t total enzyme
E_f uncombined enzyme
EI enzyme-inhibitor complex
ES enzyme-substrate complex
S_t total substrate
S_f uncombined substrate
P reaction product

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K_s dissociation constant of enzyme-substrate complex
I inhibitor
k velocity constant
v velocity of non-inhibited reaction $S \rightarrow P$
v_i velocity of inhibited reaction
a fractional activity of non-inhibited reaction $\equiv \frac{(ES)}{(E_t)}$
a_i fractional activity of inhibited reaction $\equiv \frac{(ES)_i}{(E_t)}$
i fractional inhibition $\equiv \frac{(EI)}{(E_t)}$

These symbols placed between brackets indicate the concentration of the substances mentioned. (ES) denotes the concentration of ES in the non-inhibited reaction, $(ES)_i$ in the inhibited reaction.

The reactions can be written as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES$$

$$ES \xrightarrow{k_3} E + P$$

From $k_1 >> k_3$ follows $v = k_3 \cdot (ES)$
According to the law of mass action

$$K_s = \frac{[(E_t) - (ES)] \cdot [(S_t) - (ES)]}{(ES)}$$

By substituting $(ES) \equiv a \cdot (E_t)$ and rearranging one obtains:

$$(S_t) = K_s \frac{a}{1 - a} + a\,(E_t) \qquad \text{(I)}$$

If (E_t) is neglected as compared to (S_t), this expression is reduced to the equation of MICHAELIS AND MENTEN

$$(S_t) = K_s \frac{a}{1 - a} \qquad \text{(Ia)}$$

It is presumed that the inhibition of the reaction is caused by partial blocking of the enzyme, the degree of blocking being independent of the substrate concentration. The derivation thus does not hold for competive inhibition.

$$E + I \rightleftarrows EI$$

According to the mass law one now obtains:

$$K_s = \frac{(E_f) \cdot (S_f)}{(ES)_i} = \frac{[(E_t) - (ES)_i - (EI)] \cdot [(S_t) - (ES)_i]}{(ES)_i}$$

After substituting $(ES)_i \equiv a_i\,(E_t)$
$(EI) \equiv i\,(E_t)$
and rearranging one obtains:

$$(S_t) = K_s \frac{a_i}{1 - a_i - i} + a_i\,(E_t) \qquad \text{(II)}$$

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In the theory of MICHAELIS AND MENTEN (neglection of (E_t)) this becomes

$$(S_t) = K_s \frac{a_i}{1 - a_i - i} \tag{IIa}$$

The measured percentual inhibition plotted in our curves is equal to

$$\frac{v - v_i}{v} = \frac{k_3 (ES) - k_3 (ES)_i}{k_3 (ES)} = \frac{a - a_i}{a}$$

By combining equations Ia and IIa one gets $\frac{a - a_i}{a} = i$, the classical result which stated that the percentual inhibition by a given concentration of inhibitor is a constant and thus independent of the activity level of the enzyme ruled by (S_t).

With the generalized equations I and II the result is quite different. It can be shown mathematically* that $\frac{a - a_i}{a}$ increases with increasing (S_t) and approaches the maximum value of i at high activity levels.

This mathematical proof cannot be given directly from the equation for $\frac{a - a_i}{a}$ as a function of (S_t), which is obtained by combining equations I and II (Equation III).

$$\frac{a - a_i}{a} = \frac{i (E_t) + \sqrt{[(E_t)(1 - i) + (S_t) + K_s]^2 - 4 (E_t)(1 - i)(S_t)} - \sqrt{[(E_t) + (S_t) + K_s]^2 - 4 (E_t)(S_t)}}{(E_t) + (S_t) + K_s - \sqrt{[(E_t) + (S_t) + K_s]^2 - 4(E_t)(S_t)}} \tag{III}$$

By considering equations I and II independently, however, it can be demonstrated that for values of (S_t) from zero to infinite a increases more steeply than a_i. As a consequence $\frac{a - a_i}{a}$ increases too, with increasing (S_t). An arbitrarily chosen numerical example is given in Table III.

TABLE III

CALCULATED PERCENTUAL INHIBITION FOR AN ENZYME WITH SPECIFIC ENZYME CONCENTRATION $\frac{(E_t)}{K_s} = 10$ (ZONE B ACCORDING TO STRAUS AND GOLDSTEIN) AND DIFFERENT SPECIFIC SUBSTRATE CONCENTRATIONS

Fractional inhibition $i = 0.2$

$\frac{(S_t)}{K_s}$	a	a_i	$\frac{a - a_i}{a} \times 100$
1	0.0901	0.0877	2.5
3	0.264	0.254	3.8
5	0.426	0.400	6.1
7	0.568	0.517	8.9
10	0.728	0.630	13.4
15	0.864	0.713	17.5
25	0.940	0.757	19.4
50	0.976	0.782	19.9

* We are indebted to Dr E. M. BRUINS (Amsterdam) for valuable mathematical assistance.

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It is of course not possible to interpret quantitatively our results obtained with very complicated systems by the foregoing theoretical treatment holding for a single isolated enzyme. In the first place (S_i), the substrate concentration in the immediate vicinity of the enzyme in the cell, cannot be put equal to the overall measurable substrate concentration, but is a yet unknown quantity depending upon a variety of complex factors. Neither one substrate concentration nor a single dissociation constant can be used. Further the observed activation phenomena of the "inhibitors" urethan and arsenite suggest a combination of different effects which cannot be separated at this moment[9]. Whether the dependence of the activation on the turnover number of the enzymes is real or only apparent as a consequence of the dependence of the inhibition upon the turnover number, cannot yet be decided. We are therefore not able, at the present moment, to give a full interpretation of all results. Further researches will be undertaken in order to separate the different effects.

BOYLAND[10] has shown that the distribution of urethan is equal in blood and tissues of the normal rat. Moreover he determined the concentration of urethan in the blood of patients and found values of about 30–40 mg/100 ml blood after repeated administration of 6 g urethan daily. We could confirm his results in both respects. If urethan is also equally distributed in human tissues, the concentration in the treatment of leukemia is 3 to $4 \cdot 10^{-3}$ *m*. The lowest concentrations in our experiments with kidney and liver mince, at which significant effects could be detected, are about ten times as great.

It is therefore not improbable that besides the general effect described in this paper, which in our opinion may not be left out of consideration, other effects depending upon differences between malignant and normal tissues contribute to the total effect of urethan or arsenite in the treatment of malignant growth. *E.g.* ACKERMANN AND POTTER[11] showed that the percentual inhibition also depends upon the concentration of the inhibited enzyme, which is in certain cases lower in tumours than in normal tissue, while BOYLAND[10] demonstrated that tumour bearing rats catabolize urethan more slowly than normal rats. It is perhaps possible to discover a general inhibitor of metabolism, which has nevertheless a strong specific effect on tumour growth, by taking into consideration the three points mentioned.

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SUMMARY

1. A hypothesis is given concerning the action of urethan and arsenite on malignant growth. Two assumptions are made:-

(a) the enzyme system responsible for energy production in malignant tumours is working at maximal rate, contrary to the corresponding enzyme system in normal tissues.

(b) a given concentration of urethan or arsenite blocks an equal part of the sensitive enzyme(s), by which a measurable effect of a small concentration of these inhibitors on the catabolism will only be obtained in case of maximal turnover rate.

2. Experiments with kidney and liver minces and with yeast have shown that indeed the inhibition caused by urethan and arsenite increases with increasing rate of respiration or fermentation.

3. If the enzyme concentration is not considered to be negligible as compared to the substrate concentration, as is done in the theory of MICHAELIS AND MENTEN, the increase of inhibition with increasing turnover number can be made plausible by enzyme kinetics for the much simplified case of one single enzyme.

References p. 605.

4. The dependence of inhibition upon the turnover number of the catabolic enzyme system in tumours is discussed in relation to other possible causes of the effect of urethan and arsenite on malignant growth.

RÉSUMÉ

1. Nous proposons une hypothèse concernant le mode d'action de l'uréthane et de l'arsénite sur la croissance maligne. Nous supposons que:

(a) le système enzymatique responsable de la production d'énergie des tumeurs malignes travaille à vitesse maximale, contrairement au système enzymatique correspondant des tissus normaux.

(b) unecon centration donnée d'uréthane ou d'arsénite bloque une partie égale de (ou des) enzyme(s) sensible(s). C'est pourquoi, un effet mesurable de petites concentrations de ces inhibiteurs sur le catabolisme ne sera atteint qu'en cas de vitesse de réaction maximale.

2. Des expériences faites avec des homogénats de rein et de foie et avec de la levure ont montré que, en effet, l'inhibition causée par l'uréthane et l'arsénite augmente lorsque la vitesse de la respiration ou de la fermentation augmente.

3. Lorsque la concentration de l'enzyme n'est pas considérée négligeable par rapport à la concentration du substrat, comme il est fait dans la théorie de MICHAELIS ET MENTEN, alors, pour le cas très simplifié d'un seul enzyme, des considérations de cinétique des enzymes permettent d'expliquer que l'inhibition augmente lorsque le "turnover number" augmente.

4. Nous discutons la façon dont l'inhibition du système enzymatique catabolique pourrait dépendre du "turnover number" tout en considérant d'autres causes possibles de l'effet de l'uréthane et de l'arsénite sur la croissance maligne.

ZUSAMMENFASSUNG

1. Eine Hypothese über die Wirkungsweise von Urethan und Arsenit bei malignem Wachstum wird formuliert. Hierzu werden zwei Annahmen gemacht:

(a) bei malignen Tumoren wirkt das Enzymsystem, das für die Erzeugung von Energie sorgt, mit maximaler Geschwindigkeit, im Gegensatz zum entsprechenden Enzymsystem in normalen Geweben.

(b) eine bestimmte Urethan- oder Arsenitkonzentration blockiert den gleichen Teil des (der) für diese Zellgifte empfindlichen Enzyms (Enzyme). Dadurch tritt ein messbarer Effekt kleiner Konzentrationen dieser Hemmstoffe auf den Katabolismus nur bei maximalem Umsatz auf.

2. Versuche mit Nier- und Leberbrei und mit Hefe bewiesen, dass die Hemmung, die Urethan und Arsenit verursachen, tatsächlich mit steigender Atmungs- oder Gärungsgeschwindigkeit ansteigt.

3. Wenn die Enzymkonzentration nicht, wie es in der Theorie von MICHAELIS UND MENTEN geschieht, als eine im Vergleich zur Substratkonzentration vernachlässigbare Grösse behandelt wird, kann die Zunahme der Hemmung mit zunehmender Wechselzahl für den stark vereinfachten Fall eines einzelnen Enzyms aus enzymkinetischen Betrachtungen plausibel gemacht werden.

4. Die Abhängigkeit des Hemmungsgrades von der Wechselzahl des katabolischen Enzymsystems in Tumoren wird in Zusammenhang mit anderen möglichen Ursachen der Urethan- und Arseniteffekte bei malignem Wachstum diskutiert.

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