CCXLVIII. THE METABOLISM OF NORMAL AND TUMOUR TISSUE

XVIII. THE ACTION OF GUANIDINES AND AMIDINES ON THE PASTEUR EFFECT

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AMONG the quaternary N compounds previously found to inhibit the Pasteur effect in animal tissues [Dickens, 1936] were representatives of specific anti-septics, particularly derivatives of quinoline, acridine and phenazine containing basic substituents. I am indebted to Sir Henry Dale for the suggestion that it might be of interest to study the metabolic action of the powerfully trypanocidal guanidine and amidine derivatives recently described by King *et al.* [1937], and to Dr Harold King for kindly supplying me with these compounds. In the following paper it is shown that among them are some of the most powerful inhibitors of the Pasteur effect.

Despite the interest attaching to guanidine in its possible connexion with tetany, observations of its effect on tissue metabolism are few. Meyerhof [1921] found that in minced frog muscle guanidine in concentration of $M/75$ and above caused a slight fall of respiration, below $M/100$ sometimes a slight increase. Banu & Gavrilescu [1934] investigated the action of guanidine and methyl-guanidine salts on the respiration of brei of pigeon brain, rat liver and muscle, and rabbit blood and nerve. They found in all cases a small inhibition of respiration; the concentration of inhibitor used (*c.* $M/100$, or more) was so high, however, that their postulated connexion of this effect with tetany appears questionable. The conditions of their experiments precluded the observation of the main effect of guanidine on brain metabolism reported here.

The only previous observations on the effect of guanidine on the Pasteur effect are those of Mendel, which according to Bumm & Appel [1932] were mentioned at a discussion of the Kaiser Wilhelm Institut für medizinische Forschung at Heidelberg in 1932: "Warburg fand z.B. den Blausäureäthylester wirksam" (on the Pasteur effect), "Mendel das Guanidin, wir wollen hinzufügen das auch Arginin die Pasteursche Reaktion unter Umständen partiell hemmen kann" [Bumm & Appel, 1932]. In a personal communication, Dr Mendel kindly informed me of his findings, several of which are confirmed and extended in the present paper: I was unable, however, under the same conditions to observe the effect of arginine mentioned by Bumm & Appel.

EXPERIMENTAL

All experiments were made at 37.5° in the Warburg apparatus, using tissue slices suspended in NaHCO_3 -Ringer solution containing 0.2% glucose and in equilibrium with $\text{O}_2 + 5\% \text{CO}_2$ or $\text{N}_2 + 5\% \text{CO}_2$. The tissue used was rat brain cortex unless otherwise stated. In order to follow the time course the Warburg 2-vessel method was used for all aerobic experiments. "0 min." was 15 min. after inserting the vessels in the thermostat.

Where necessary the added substances (usually hydrochlorides) were neutralized before use; generally the dilutions were high enough for this to be immaterial, and for the same reason retention of CO_2 by the medium was negligible. However, with extremely high dilutions ($10^{-6} M$) a technical difficulty not previously experienced became evident; the absolute amount of inhibitor contained in the vessel with the smaller volume of solution was evidently insufficient to saturate the tissue, while in the larger volume normal inhibition occurred. This indicates either absorption or chemical reaction of the guanidine with some tissue component; it was avoided to some extent by taking tissue weights approximately in proportion to the liquid volumes. This also has the advantage of giving similar amounts of lactic acid per ml. Ringer solution in the two vessels. The effect of this phenomenon would be to give false low values for respiration and aerobic glycolysis, hence it could not have entered significantly with concentrations higher than about $10^{-6} M$, which caused increased respiration and glycolysis.

Guanidine. Guanidine carbonate (A.R., British Drug Houses) was dissolved directly in the Ringer solution. (The term "molar" refers throughout to the content of guanidine or other bases.) Addition of guanidine salt in $10^{-3} M$ concentration completely inhibits the Pasteur effect in rat brain slices suspended in Ringer solution or serum (Table I). The respiration is unchanged or increased; the aerobic glycolysis is greatly increased and may exceed the anaerobic glycolysis

Table I. *Action of guanidine on metabolism of rat brain cortex*

Guanidine	Time (min.)	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Medium: Ringer solution				
Nil	0-120	12.0	1.5	15
$10^{-3} M$	0-30	17.9	26.4	—
	30-60	16.3	26.8	—
	60-90	20.5	26.2	—
	90-120	18.9	22.0	—
$5 \times 10^{-5} M$	0-30	11.9	4.8	—

(Anaerobic glycolysis with $10^{-3} M$ guanidine present from start; 0-60 min. 125%, 60-120 min. 140% of control without guanidine.)

Medium: inactivated horse serum				
Nil	0-60	10.6	3.0	—
$10^{-3} M$	0-30	12.7	15.7	—
	30-60	13.4	17.3	—

Lactic acid estimation: Ringer solution, $O_2 + CO_2$. Chemical estimation by method of Friedemann & Kendall, after Cu-Ca(OH)_2 treatment. Duration 100 min. (Owing to large weight of tissue relative to NaHCO_3 in the experiment with guanidine the rate of aerobic acid formation fell off during the experiment.) Simultaneous manometric observations were used to calculate total acid formation of the same tissue.

		Controls		With guanidine 10 ⁻³ M
Tissue dry wt. (mg.)	...	8.56	16.19	14.36
Chemical estimations:				
Lactic acid, final (mg.)		0.322	0.542	1.202
Lactic acid, preformed (mg.)		0.057	0.107	0.135
Lactic acid formed (mg.)		0.265	0.435	1.067
Manometric estimations:				
Total acid formed, calc. as lactic (mg.)		0.248	0.469	1.04
Chemical Manometric × 100		106 %	93 %	98 %

of the control; the anaerobic glycolysis is often at first increased but later falls slowly. At a concentration of 5×10^{-5} *M* guanidine has no appreciable effect. Chemical estimation of the aerobic lactic acid shows that it accounts for practically the whole aerobic acid formation (Table I).

Table II. *Reversal of guanidine action*

Rat brain cortex. $O_2 + CO_2$. $NaHCO_3$ -glucose-Ringer solution. 37.5° . Guanidine conc. 10^{-3} *M*.

Time min.	Medium	Q_{O_2}	$Q_G^{O_2}$	Medium	Q_{O_2}	$Q_G^{O_2}$
0-20	Guanidine-Ringer	19.2	18.3	Plain Ringer	15.1	2.8
	(Transferred to fresh Ringer)				(Continued)	
40-60	Plain Ringer	11.0	6.3	"	13.7	2.5
60-75		13.5	5.6		9.9	2.5
75-90		12.5	4.1			
90-110		11.6	1.9		7.5	2.4

(Control with 10^{-3} *M* guanidine present throughout: $Q_G^{O_2}$ mean over 2 hr. = 18.2.

Table III. *Action of guanidine on various cells*

Medium: animal tissues, Ringer- $NaHCO_3$ -glucose; yeast, $M/20$ KH_2PO_4 -glucose.

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2}$
Rat amnion:			
Guanidine 10^{-3} <i>M</i>	8.6	6.1	12.6
Control	6.4	5.7	11.6
Guinea-pig medulla of kidney:			
Guanidine 10^{-3} <i>M</i>	7.6	12.5	30.0
Control	9.5	8.4	25.8
Jensen rat sarcoma:			
0-30 min. no guanidine	9.0	28.6	31.3
At 30 min. tipped in guanidine from side bulb to make 10^{-3} <i>M</i> :			
40-60 min. with guanidine	7.1	26.2	31.3
Baker's yeast:			
Guanidine $M/500$	51.4	42.7	133
Control	55.5	20.4	150

The action of guanidine on rat brain is reversed merely by transferring the tissue to guanidine-free Ringer solution (Table II). The reversal is not instantaneous but is complete about 50 min. after transference. This suggests a slow dissociation of some complex formed by the guanidine; it can hardly be merely a slow diffusion out of the tissue, for the inward path is rapid as shown by the rapid inhibition of the Pasteur effect which occurs within a few minutes of the addition of guanidine.

These effects of guanidine are absent or less marked in other tissues tested (Table III).

Methylguanidine, *as-dimethylguanidine*, *agmatine*. Methylguanidine HCl (B.D.H.) acts like guanidine, *as-dimethyl guanidine* HCl (Roche) shows only slight activity in inhibiting the Pasteur effect, and both substances poison the respiration slowly. Agmatine (δ -aminobutylguanidine), used as the sulphate (Roche), differed from the other compounds studied in that the aerobic glycolysis steadily increased during the experimental period of $2\frac{1}{2}$ hr. (Table IV). It is of interest that arginine, which yields agmatine on decarboxylation, did not inhibit the Pasteur effect.

Table IV. *Methylguanidine, as-dimethylguanidine, agmatine*Rat brain cortex. NaHCO_3 -Ringer-glucose. $\text{O}_2 + \text{CO}_2$. 37.5° . Tissue from 2 rats mixed.

Time (min.)	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$
	Control (no addn.)		<i>as</i> -Dimethylguanidine $10^{-3} M$	
0- 60	12.8	2.4	12.1	6.5
70- 90	11.9	2.0	5.3	7.8
90-120	13.3	1.6	4.9	4.1
	Methylguanidine $10^{-3} M$		Agmatine sulphate $10^{-3} M$	
0- 60	11.1	19.3	13.8	5.8
70- 90	8.8	21.0	15.0	8.0
90-120	5.8	16.6	13.2	10.3

Table V. *Guanido-derivatives, biuret, urea and acetamide*Rat brain cortex. NaHCO_3 -Ringer-glucose. $\text{O}_2 + \text{CO}_2$. 37.5° .

Time (min.)	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$
	Control (no addn.)		Creatine $10^{-3} M$		Arginine $10^{-3} M$	
0- 30	11.6	1.9	5.9	2.7	6.9	2.4
30- 60	11.1	0.3	3.2	0	2.8	0.0
60-120	11.1	0.6	1.9	0.1	1.0	0.0
	Control (no addn.)		Glycocyamine $10^{-3} M$		Biuret $10^{-3} M$	
0- 30	15.8	5.0	13.3	4.8	12.4	1.5
30- 60	14.9	2.5	7.9	2.2	12.3	-0.2
60-120	10.2	2.6	6.0	2.3	11.3	0.1
	Control (no addn.)		Acetamide $M/50$		Urea $M/50$	
0- 20	12.1	4.4	11.7	3.3	12.9	3.2
20- 60	11.9	2.8	11.2	1.6	13.0	3.2

Inactive compounds. Among compounds of similar structure to the above, the guanido-derivatives arginine, creatine and glycocyamine were all rather surprisingly toxic to the respiration without any inhibition of the Pasteur effect such as is shown by guanidine itself. All the above were tested in $10^{-3} M$ solution. At the same concentration biuret, and in $M/50$ solution urea and acetamide, had no effect whatever (Table V).

Decamethylenediguandine and 1:11-undecanediamidine. These two substances, used as dihydrochlorides, showed quite remarkable activity in inhibiting the Pasteur effect (Table VI). With the higher concentrations (8×10^{-4} and $5 \times 10^{-5} M$) the metabolism became very much increased and unstable, particularly the aerobic glycolysis which rose to an unusual height for the tissue. This effect is reminiscent of that of disturbance of the ionic balance of the medium [Dickens & Greville, 1935] with the difference that the increase extends even to the anaerobic glycolysis, which always falls below normal when salt effects are the cause of aerobic glycolysis. Whatever may be the explanation of the guanidine effect, it cannot very well be due to a different permeability aerobically and anaerobically, such as has been assumed to be the basis of the salt effect.

For the reason mentioned in the introduction to the experimental part, the upper limit of dilution which is active is difficult to determine. $10^{-6} M$ solutions were sometimes active, sometimes not. One factor is undoubtedly the ratio weight of tissue : weight of guanidine or amidine compound (Table VI). For a substance of mol. wt. 330, a $10^{-6} M$ solution contains $0.33 \mu\text{g.}$ per ml. or 1 part per 3 million. This amount is of the same order as that of vitamin B_1 required to produce the catatorulin effect in avitaminous pigeon brain [Peters, 1937]. It is

Table VI. *Decamethylenediguanidine (Synthalin), 1:11-undecanediamidine, etc.*

Rat brain cortex. NaHCO_3 -Ringer-glucose. 37.5° . Zero time = 15 min. after putting in the bath.

Substance	Conc. <i>M</i>	Time min.	With substance			Control		
			Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$	$Q_{\text{G}}^{\text{N}_2}$	Q_{O_2}	$Q_{\text{G}}^{\text{O}_2}$	$Q_{\text{G}}^{\text{N}_2}$
Decamethylene- diguanidine	8×10^{-4}	0- 30	22.6	37.6	—	13.3	4.3	—
		30- 60	16.6	25.5	—	11.4	1.2	—
		60- 90	12.6	18.8	—	10.7	1.8	—
		90-120	7.2	14.6	—	10.9	0.5	—
	5×10^{-5}	0- 20	16.2	25.2	23.0	15.7	1.5	13.4
		20- 40	12.6	26.1	20.6	15.6	0.7	12.6
		40- 60	10.3	27.2	20.0	15.6	0.4	12.3
		60- 80	8.5	10.7	18.2	13.9	0.3	11.8
	10^{-6} (7 mg. dry wt. tissue)	0- 90	(12.0)*	22.1	—	13.4	2.0	—
	10^{-6} (14 mg. dry wt. tissue)	0-120	14.8	2.8	—	10.8	1.2	14.2
1:11-Undecane- diamidine	8×10^{-4}	0- 60	2.4	22.3	—	12.3	2.7	—
		60-120	1.9	15.0	—	10.8	1.1	—
	5×10^{-5}	0- 60	10.4	20.3	15.7	9.8	1.1	15.4
		60-120	11.0	21.5	13.8	9.7	1.3	13.1
	10^{-6}	0- 60	(12.0)*	25.0	—	9.8	1.1	15.4
Decamethylene- diisothiourea	8×10^{-4}	0- 60	16.8	12.0	—	12.3	2.7	—
		60-120	7.8	3.9	—	10.8	1.1	—
	5×10^{-5}	90-120	9.6	9.6	—	9.5	0.9	13.2
Hexadecamethylene- diguanidine	Satd.	0- 60	12.6	4.4	—	15.6	1.2	12.8
	(< 4×10^{-4})	60- 80	8.4	3.9	—	13.9	0.3	11.8

* Approx. only with 10^{-6} *M* conc.: see text.

evident, therefore, that the effects described in this paper are catalytic or anti-catalytic.

King *et al.* [1937] found that the trypanocidal activity *in vivo* of polymethylene diguanidines increased with the length of the C-chain, being low for the 2 to 6-C members, becoming first apparent with the octamethylenediguanidine and increasing in the 10 to 14-C compounds. It is of interest to note that on the Pasteur effect guanidine itself has only about 1/1000th of the activity of decamethylenediguanidine. The only higher member of the series tested, hexadecamethylenediguanidine, was however almost insoluble in the saline medium used, being only partially dissolved when added in 4×10^{-4} *M* concentration; consequently the actual amount present in solution was unknown. Under these conditions it increased aerobic glycolysis but slightly (Table VI).

Decamethylenediisothiourea, found by King *et al.* [1937] to be trypanocidal only *in vitro*, showed a fairly well-marked guanidine effect in 5×10^{-5} *M* solution: in higher concentration (8×10^{-4} *M*), after an initial effect it proved toxic to both respiration and glycolysis. This compound was used as the dihydrobromide (Table VI).

Inactive compounds related to the above group. Acetamide itself in 10^{-3} *M* solution poisons both respiration and glycolysis: in lower concentration (10^{-5} *M*) it is without effect (Table VII). Here again the presence of the long-chain substituent appears necessary both for trypanocidal activity [King *et al.* 1937] and for inhibition of the Pasteur effect by high dilutions.

2:7-Naphthalenediamidine and *pp'*-diguanylidodiphenylmethane were also inactive in 5×10^{-5} *M* concentration (Table VII); here the incorporation of the polycarbon chain in aromatic ring systems appears to destroy activity, but it is possible that this might be due to inability to permeate the cell.

Table VII. *Inactive compounds related to those of Table VI*

Substance	Conc. <i>M</i>	Time min.	With substance		Control	
			Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
Acetamidine	10^{-3}	0-30	5.5	3.2	16.9	3.8
		30-60	3.6	1.1	15.1	2.1
	10^{-5}	0-60	16.1	3.4	16.0	3.5
2:7-Naphthalenediamidine	5×10^{-5}	0-120	14.3	2.4	14.3	3.7
<i>pp'</i> -Diguanylidodiphenylmethane	5×10^{-5}	0-120	12.3	2.8	14.3	3.7

Table VIII. *Various trypanocidal substances and related compounds*

Substance	Conc. <i>M</i>	Time min.	With substance		Control	
			Q_{O_2}	$Q_G^{O_2}$	Q_{O_2}	$Q_G^{O_2}$
Germanin (Bayer 205)	10^{-4}	0-120	10.9	2.1	11.1	1.5
Trypan blue	10^{-3}	0-30	11.1	4.0	12.6	2.9
		30-60	10.3	1.6	10.6	2.1
		0-30	9.4	4.8	As above	
Rivanol	5×10^{-5}	30-60	5.8	4.4		
Gonacrine (diaminoacridine metho- chloride)	5×10^{-5}	0-120	17.7	21.6	14.3	3.4
<i>p</i> -Aminophenyl-dichloroarsine	10^{-3}	0-60	0.1	0.2	11.6	2.5
Picric acid	10^{-3}	0-60	11.8	0.6	11.6	2.5
Dinitro- <i>o</i> -cresol (same tissue as 1st exp. of Table I)	5×10^{-5}	0-20	24.7	9.0	11.8	2.8
		20-40	17.0	10.4	12.1	2.4
		40-60	7.4	6.1	11.5	0.9

Trypanocides not chemically related to guanidine. In addition to the above two compounds, a number of well-known trypanocides (Table VIII) proved to have no effect on brain metabolism. Thus Trypan blue (formed by coupling diazotized *o*-toluidine and H-acid), picric acid (10^{-3} *M*) and Rivanol (5×10^{-5} *M*) were quite without action, while *p*-aminophenyldichloroarsine (10^{-3} *M*) had an almost instantaneous and complete poisoning effect.

Similarly Germanin (Bayer 205) in *c.* 10^{-4} *M* concentration had no effect on metabolism over 2 hr. In this case, the lack of activity might reasonably be ascribed to impermeability of the tissue, for Germanin has mol. wt. = *c.* 1500 and contains 6 sulphonic acid groups. Such an explanation however could not account for the non-activity of Trypan blue, which was observed to stain the brain slices deeply and uniformly.

The inertness of picric acid (used as Na salt) is worthy of note; for comparison one observation with dinitro-*o*-cresol is included in Table VIII, which confirms the effect of this compound on brain metabolism observed by Greville [1939]. It may be that the optimum concentration was not used, but in the experiment quoted the initial increase of respiration was the most striking effect and the clearness with which the Pasteur effect was inhibited lags far behind the experiment with guanidine and the same sample of tissue given in Table I. Dinitro-*o*-cresol shows some activity against relapsing fever spirochaetes [Fischl, 1934].

Diaminoacridine methochloride ("Gonacrine") is evidently one of the class of inhibitors of the Pasteur effect already described [Dickens, 1936], and is of similar activity to the rest of this group. On the other hand, Rivanol, containing

no quaternary N, has no appreciable effect on the Pasteur reaction, as appears to be the general rule with such compounds [Dickens, 1936].

Action of amidine compounds on carboxylase. Axmacher & Bergstermann [1934] showed that the action of yeast carboxylase on pyruvic acid was inhibited by Germanin. This effect, however, is not shown by substances containing the amidine group which are known to be trypanocidal (Table IX). Hence this action does not appear to be characteristic either of trypanocides or of inhibitors of the Pasteur effect.

Table IX. *Action on yeast carboxylase*

Each vessel contained in the main compartment 50 mg. carboxylase [Axmacher & Bergstermann, 1934] in 0.5 ml. *M*/20 phosphate buffer pH 6.2 together with 1.5 ml. 0.9% NaCl or 1.5 ml. *M*/200 substance in 0.9% NaCl. The side bulb held 0.2 ml. *M*/10 Na pyruvate. Final conc. of added substance c. *M*/300. Temp. 25°. Gas space, air. Decarboxylation was virtually complete in 30 min. in the control.

CO₂ evolved as % of control without added substance:

CO ₂ (Control = 100) after min.	Addition				
	Germanin	Hexadeca- methylene- diguandine	1:11-Undecane- diamidine	Deca- methylene disothiurea	Synthalin
5	66	99	102	98	101.5
10	79	97	97	95	98
15	85	97	95.5	94	97
20	86.5	97	95	93	96.5
30	89	100	93.5	92.5	96

DISCUSSION

Inhibition of the Pasteur effect. Like the action of cations, i.e. either excess K⁺ [Ashford & Dixon, 1935] or disturbance of physiological balance due to lack of K⁺ and Ca⁺⁺ [Dickens & Greville, 1935], or the presence of NH₄⁺ [Weil-Malherbe, 1938], that of the guanidine compounds described in this paper appears to be limited to, or most marked in, cerebral tissue. Possible reasons for this have already been discussed by Dickens & Greville [1935] and Dixon & Holmes [1935]. A further peculiarity of brain slices is that, owing to the anatomical structure of the brain, the preparation of the tissue slice must involve the cutting of numerous nerve-fibres and axons; hence in slices of cortical tissue, unlike those of the epithelial organs, almost every cell will present an injury-surface and this may cause an increased susceptibility to the composition of the suspension medium. While this may exaggerate an existing tendency it appears likely from the correspondence of *in vivo* effects with metabolic activities of the slice, that the *in vitro* actions of added substances such as those recorded here are not to be regarded as mere artefacts, but may well have real physiological significance. The present series of compounds is admirably suited for experimental investigation of their *in vivo* effects on brain metabolism, and it is hoped, in view of the often suggested connexion of guanidine compounds with tetany, that physiologists will examine the lactic acid content of brain tissue in guanidine and other forms of tetany.

As to the mechanism of action, no clear interpretation is possible at present. Two possible theories may be tentatively suggested, though neither is susceptible of proof. These are (1) depression of the ionization of Ca, (2) reaction of guanidine with tissue protein, resulting in its denaturation and probably in the liberation of SH groups. Apart from the inconclusive nature of the depression of ionization

or "inactivation" of Ca by guanidine derivatives [Frank *et al.* 1921; Thomson & Collip, 1932], in the present experiments the amount of guanidine derivative required is very much less than that of Ca^{++} present. The Ringer solution contained $M/500$ Ca^{++} , and only half this concentration of guanidine and 1/200th of this quantity of synthalin were necessary to inhibit completely the Pasteur effect in brain. It is, of course, possible that the inactivation of catalytically active amounts of Ca^{++} present at enzyme surfaces in the brain tissue could be caused even by these low concentrations. With regard to (2), it is well recognized that agents producing denaturation of protein are capable of causing irreversible inhibition of the Pasteur effect, which is in general the first metabolic sign of cell injury. Heat¹ [Kubowitz, 1929; Dickens *et al.* 1936; 1937; Dixon, 1936], or the use of Ringer solution instead of serum as a suspension medium for certain delicate tissues [Negelein, 1925] may cause inhibition of the Pasteur effect accompanied by a whitish appearance of tissue which probably indicates coagulation of protein. From a study of the published data it seems possible that the first metabolic effect of radium *in vivo* [Crabtree, 1932] or of X-rays *in vitro* [Bancroft & Kinsey, 1937] may have been irreversible injury to the Pasteur mechanism.

Such a destructive mechanism might act in several ways. It might bring about the direct destruction of some special "catalyst" of the Pasteur reaction such as Warburg [1926] supposed to exist; this compound would therefore be a highly thermolabile enzyme, since its irreversible inactivation proceeds at temperatures not exceeding 45° [Dickens *et al.* 1936; Dixon, 1936].

It has often been claimed that SH compounds may play an important part in regulating aerobic glycolysis [Quastel & Wheatley, 1932; Rapkine, 1931; 1938; Bumm & Appel, 1932; see however Baker, 1937; Weil-Malherbe, 1938]. Denaturation of proteins is frequently accompanied by liberation of SH groups, and it is possible that this property may be involved in the mechanism of the Pasteur reaction. One could imagine the "catalyst" of the Pasteur effect to be an enzyme having as its active group a protein -S-S linkage, which on denaturation would be transformed into the SH group. It is admittedly difficult to imagine that normally the mere transference of the tissue from aerobic to anaerobic conditions should be capable of causing such a change, which must, moreover, be reversible; Rapkine [1937] has however put forward a similar view of the changes in cellular division, which he supposes to be preceded by denaturation of protein. Without necessarily subscribing to these views, it may be pointed out that guanidine compounds are able to bring about denaturation of proteins [Greenstein, 1938; 1939] and their activity is considerably in excess of that of urea, amides etc. [Hopkins, 1930], as judged by the rate and extent of appearance of SH groups. The effect on the Pasteur reaction of the substances described in this paper presents a number of points of similarity with what might be expected from the resonance theory of Greenstein [1938]. For instance, methylguanidine and guanidine ions, which both show resonance and denature proteins, both inhibit the Pasteur effect; while the *as*-dimethylguanidonium ion does not appreciably inhibit the Pasteur effect, its resonance is greatly restricted, and it is a much less effective denaturing agent. (I have attempted to determine the denaturing ability towards ovalbumin of decamethylenediguanidine and 1:11-undecane-

¹ Dixon [1936] considered that in brain tissue there was not a true inhibition of the Pasteur effect, but only an apparent one caused by the greater rate of fall of anaerobic than of aerobic glycolysis at 45°. However, in the experiments of Dickens *et al.* [1936] this rapid fall was often entirely absent, and Dixon's objection appears to us therefore not to apply to our observations, which were submitted for publication simultaneously with and independently of those of Dixon.

diamidine, the two most active inhibitors of the Pasteur reaction, but unfortunately their low solubility made this impossible except perhaps by the use of a very readily denatured protein.) The concentrations of guanidine compounds used by Greenstein were very much higher than those which inhibit the Pasteur effect, but one might expect the protein concerned in the latter to be more readily denatured than those employed by Greenstein. The most serious objection to this theory is the lack of any effect on metabolism of urea and acetamide, even in high concentrations; moreover, it is not known whether reversible denaturation can occur with guanidine compounds.

Specific antiseptics. The parallelism between antiseptic and metabolic activity, which up to a point is striking for chemically similar substances, breaks down when a wider variety of compounds are considered. Thus although certain quinoline and acridine antiseptics [Dickens, 1936], which are chemically quite unlike the guanidine compounds here described, show the same inhibition of the Pasteur effect in brain tissue, there are a number of powerful trypanocides which do not affect brain metabolism (e.g. Trypan blue, which stains the tissue deeply without affecting its respiration and glycolysis at all). It is not impossible, however, that the extension of these observations to the more complicated metabolism of the trypanosome might lead to results of value in the understanding of specific antiseptics: the most striking feature of the metabolism of *Trypanosoma Rhodesiense*, studied by Christophers & Fulton [1938], appears to be the large utilization of glucose accompanied by formation of acid. Since the respiration is known to be very sensitive to acid, an increased aerobic acid production might be of significance. However, the R.Q. of the trypanosome is only 0.2 and since the products formed from glucose are unknown there is little apparent resemblance between the metabolism of the parasite and of brain tissue.

Tetany. The similarity between the tetanic convulsions of guanidine poisoning and those following parathyroidectomy has led to numerous theories attributing tetany to intoxication with guanidine or its methyl derivatives; these are critically discussed by Thomson & Collip [1932].

It is by no means clear if the present observations have any direct connexion with tetany: it may however be remarked that in brain tissue the effects of absence of Ca^{++} from the medium [Dickens & Greville, 1935] or of addition of guanidine compounds are in the same direction, both causing increased respiration and aerobic glycolysis. On the other hand, dimethylguanidine, which has frequently been suggested as a cause of tetany, is much less active on the Pasteur effect than guanidine itself or its monomethyl derivatives.

Banu & Gavrilescu [1934] consider that the slight depression of brain respiration observed by them in the presence of high concentrations of guanidine may be, at least in part, the cause of guanidine tetany. It seems to the author that, if the effects described in this paper also occur *in vivo*, they might provide a far more probable explanation of guanidine tetany. The excessive aerobic acid production and unstable high metabolism observed in brain tissue, resembling in type that caused by lowered Ca^{++} content of the medium, would appear to be an action more likely to produce drastic pathological effects: it occurs moreover with concentrations of guanidine compounds which might not inconceivably be present in the animal organism in tetany.

SUMMARY

Addition to the medium of $M/1000$ guanidine completely and reversibly inhibits the Pasteur effect in slices of rat brain cortex, the respiration continuing usually at a higher level while the aerobic (lactic) acid formation attains or

exceeds the anaerobic. Complete restoration occurs on transference to guanidine-free medium. These effects are absent or less marked in other tissues and cells (amnion, medulla of kidney, Jensen sarcoma, baker's yeast). Lower concentrations ($5 \times 10^{-5} M$) are ineffective. Methylguanidine acts like guanidine, but the dimethyl derivative shows little typical action. Glycocyamine, arginine and creatine ($10^{-3} M$) are toxic; agmatine causes a slow inhibition of the Pasteur effect. Biuret ($10^{-3} M$) and $M/50$ urea and acetamide have no action on respiration or glycolysis.

Certain substituted guanidines and amidines are the most powerful inhibitors of the Pasteur effect yet described: these are 1:11-undecanediamidine and decamethylenediguanidine (Synthalin), which are fully active at 10^{-6} – $10^{-5} M$, the precise concentration depending on the relative amounts of solution and tissue. Both are powerful trypanocides; other specific antiseptics of the acridine and quinoline series have previously been found to inhibit the Pasteur effect in brain. This property is not however shared by all trypanocides, for Bayer 205 ($10^{-4} M$) and Trypan blue ($10^{-3} M$) do not affect brain metabolism. Decamethylenediisothiurea causes an incomplete inhibition of the Pasteur effect.

The bearing of these results on the mechanism of the Pasteur effect, and the possibility of their connexion with tetany and specific antiseptics are discussed.

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