The Effect of Arsenical Vesicants on the Respiration of Skin

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It has been known for many years that trivalent compounds of arsenic are potent inhibitors of certain enzyme systems, including some of those involved in cell respiration. Among the arsenic-sensitive enzymes are those responsible for the oxidation of α-keto-acids (Krebs, 1933). It is now known that compounds of arsenic are highly selective in their action in low concentrations on cell enzymes, and it is believed that the pyruvate oxidase enzyme system is prominent among those affected (Peters, 1936; Sinclair & Thompson, 1940); the evidence for this is based both on the high sensitivity of the pyruvate oxidase system of brain to trivalent arsenicals *in vitro*, and on the *in vivo* finding of a rapid rise in the level of the blood pyruvate in animals poisoned with arsenic, analogous to that occurring in vitamin B_1 deficiency (Thompson & Johnson, 1935).

One possible mechanism for initiating skin damage by the arsenical vesicants, leading ultimately to the formation of a vesicle, would be the production by the arsenical of some metabolic disturbance in the skin, such as the blocking of pyruvate oxidation due to the vesicant combining with or in some way inactivating the enzyme concerned. The background of this 'enzyme theory' of vesication, and the facts on which it is based are discussed in an earlier paper (Peters, Sinclair & Thompson, 1946).

It was important therefore to determine whether arsenical vesicants exert the same inhibitory action on the respiration of skin as on that of brain tissue, and whether a correlation exists between the development of visible pathological changes in the skin (erythema or oedema) and any specific inhibitions of oxidations in the skin. Following on the earlier work with brain, a comparison has therefore been made of the respiration of normal skin and of skin to which lewisite had been applied during life, and also of the specific oxidations of pyruvate and succinate in skin under these conditions.

Owing to the relatively large amount of inert tissue in mammalian skin (keratin and elastic fibrous tissue), the respiratory activity of whole skin is low. Thus, Wohlgemuth & Klopstock (1926) found a mean Q_{O_2} (μl. O_2/hr./mg. dry wt.) of 2·1 for human skin obtained from amputated limbs, and for normal skin obtained from plastic operations; a mean value of 1·48 was reported by Amersbach, Nutini & Cook (1941) for human skin taken from below the axilla in the mid-axillary line. Buhmann (1936) obtained an even lower mean value of 1·38 (ranging from 0·5 to 2·5) for normal human skin. Adams (1936), working with adult rat skin, found values ranging from 0·56 to 2·5, while Cook, Kreke & Nutini (1938) obtained values varying from 0·5 to 1·15.

The age of the animals has a marked effect on the respiratory activity of the skin, due probably to variations in the thickness of the keratin layer; thus, Loebel (1925) found for the skin of newly born white mice a Q_{O_2} as high as 6·9, and for the skin of one newly born rat a Q_{O_2} of 3·5. Needham & Dixon (1941), using 2–5-day-old rats, obtained values of the order of 4–5, while Adams (1937) showed that a steady fall in respiratory rate occurred from 10 to 210 days.

The effect of inhibitors on the respiration of skin appears to have received little attention. Huf (1936, 1938), however, has shown that 0·001 M-KCN brings about a substantial decrease in the oxygen uptake of frog's skin. Francis & Gatty (1938) have studied the effect of iodoacetate on the respiration of frog's skin, and have attempted to correlate the inhibition of oxygen uptake with the decrease also brought about by iodoacetate in the electrical p.d. across the skin. It is interesting to note that these workers also observed that arsenite brings about a decrease in the electrical p.d. of skin; arsenite was only used in a few experiments, however, and no details are given.

EXPERIMENTAL

Estimation of enzymic activity. Oxygen consumption was determined manometrically, the skin being sliced and suspended in a Ringer-phosphate solution (containing calcium) at pH 7·3 and at 38°. Owing to the low rate of respiration of skin, between 200 and 300 mg. of skin slices were added to each bottle, and the oxygen uptake measured in duplicate over two successive 1 hr. periods. The initial difficulty experienced in drying the skin slices down to constant weight necessitates the expression of results as μl. O_2/g. tissue (wet wt.)/hr., the mean value throughout the 2 hr. periods of the experiments being given.

Substrates and inhibitors. Two substrates, sodium pyruvate (kindly supplied by Prof. R. A. Peters) and sodium succinate, were used, the final concentrations in the bottles being 0·02 and 0·06 M respectively.

Sodium arsenite and lewisite (kindly supplied by the Ministry of Supply Experimental Station, Porton) were used as inhibitors, and, when added direct to the bottles, the final concentrations were 10^{-4} and 3×10^{-5} M respectively. The sodium arsenite was prepared by dissolving arsenious oxide (British Drug Houses Ltd., A.R.) in water by warming and with the addition of N-NaOH, subsequently adjusting the pH to 7·3. The lewisite was dissolved directly in Ringer-phosphate.

Source of tissue. Rat skin has been used throughout this work. In the earlier experiments adult rats were used; in later experiments young rats (3–4 weeks old) were selected in order to obtain a larger oxygen uptake *in vitro*, while in a few experiments (see Table 2), following the work of Needham & Dixon (1941), baby rats (2–3 days old) were used.

The skin slices were obtained as follows: The rats were first lightly anaesthetized with ether, and a large area of skin on the belly and flanks clipped free from fur. The clipped area was then washed with ethanol and water. Two hours after the end of the anaesthetic, or longer, the animal was killed by decapitation, the skin removed, scraped free from subcutaneous tissue and sliced with a razor.

RESULTS

Respiration of normal skin in the presence of arsenite or lewisite

The effects of the addition of sodium arsenite on the 'residual' respiration (i.e. in the absence of added substrate) of normal skin, and on respiration in the presence of added pyruvate were first studied. Since it has been shown by Sinclair & Thompson (1940) that the succinoxidase system of brain is relatively unaffected by concentrations of arsenite that cause an almost complete inhibition of pyruvate oxidation by brain, succinate has also been used as a substrate in order to determine whether arsenite in low concentrations also exerts a selective action on the respiratory enzymes of skin, or whether there is in this tissue a more generalized inactivation of cellular function.

The values obtained for the oxygen uptake of skin slices respiring in Ringer-phosphate solution agree well with those of earlier workers. The addition of 0·02 M-pyruvate to the medium results in a slight but definite increase in oxygen uptake. Succinate is oxidized more rapidly.

In Table 1 may be seen a series of values for the *extra* oxygen uptake brought about by the addition of these substrates to *adult* rat skin slices. One experiment is purposely included in Table 1 in which added pyruvate caused no extra oxygen uptake; a further example is given in Table 3. Such experiments have very occasionally occurred throughout the work on adult skin.

It might be argued that the small and variable extra uptake produced by added pyruvate is not due to oxidation of the pyruvate but to a catalytic effect produced by the pyruvate on some other oxidation system. Sellei & Spiera (1938), however, have shown that the skin normally stores carbohydrate, so that this store may be adequate to maintain the respiration at an almost maximum rate in the absence of any added carbohydrate substrate; it should be noted in this connexion that glucose also brings about only a small extra uptake. Moreover, in view of the central role that pyruvate is now believed to play in carbohydrate metabolism, it is thought likely on present evidence that true oxidation of pyruvate by skin does occur. Peters & Wakelin (1943) brought forward further evidence for this by demonstrating the presence of co-carboxylase in rat skin.

Table 1. *Extra respiration of normal adult rat skin slices due to the presence of added substrates*

	(μl. O_2/g./hr.)	
	Pyruvate	Succinate
	28	104
	20	91
	36	164
	95	137
	45	224
	12	78
	0	123
	49	87
Mean	36	126

The effects produced by arsenite and lewisite on these oxidations are shown in Table 2. In these experiments the arsenical was added to the Ringer-phosphate solution in the bottles (with or without extra substrate), the skin slices added next and their weight determined by re-weighing the bottles. It will be seen that in every case the addition of the arsenical caused an inhibition of the residual respiration and also of the total respiration in the presence of added substrate. When calculated for the extra oxygen consumption due to the added substrate, however, it will be seen that while in every experiment but one pyruvate oxidation has been markedly inhibited (86–100 %), succinate oxidation has been only slightly affected (0–19 %).

In most cases the respiration was well sustained and the inhibition produced by the arsenical was approximately constant over the two successive periods of 1 hr.

The respiration of lewisite-contaminated skin

It was next of interest to determine whether similar biochemical derangements were present in skin to which an arsenical vesicant had been applied during life.

The procedure adopted was as follows: Male piebald rats, 4–8 months old, were selected. Two large areas of skin (roughly 30 sq.cm.) on either side of each animal were clipped and shaved while

Table 2. *Effect of added arsenicals on pyruvate and succinate oxidation by normal rat skin slices*

(Results as μl. O_2/g./hr.)

Pyruvate

				Inhibition (%)		Extra O_2 uptake in presence of substrate		
R	*RA*	*P*	*PA*	*R*	*P*	(*P*-*R*)	(*PA*-*RA*)	Inhibition (%)
			Arsenite. Adult rats					
112	75	143	76	33	47	31	1	97
124	67	158	58	56	63	34	0	100
200	137	213	138	32	35	13	1	92
170	98	200	143	42	29	30	45	0
129	98	159	91	24	43	30	0	100
148	73	169	76	51	55	21	3	86
141	72	161	66	49	59	20	0	100
149	128	176	112	14	36	27	0	100
172	131	215	114	24	47	43	0	100
135	112	185	115	17	38	50	3	94
			Lewisite. Baby rats					
—	—	399	205	—	49	—		—
—	—	408	171	—	58	—	—	—

Succinate

				Inhibition (%)				
R	*RA*	*S*	*SA*	*R*	*S*	(*S*-*R*)	(*SA*-*RA*)	Inhibition (%)
			Arsenite. Adult rats					
148	73	291	205	51	29	143	132	8
141	72	303	206	49	32	162	134	17
149	128	349	289	14	17	200	161	19
168	139	303	262	17	14	135	123	9
148	74	237	206	50	20	109	132	0

R = residual.
RA = residual + arsenical.
P = pyruvate.
PA = pyruvate + arsenical.
S = succinate.
SA = succinate + arsenical.

the animal was under light ether anaesthesia. Ethanol (0·2 ml.) was dropped from a pipette on to the shaved area of one side, and was spread over the area with a glass rod; 0·2 ml. of a suitable dilution of lewisite in ethanol was then spread over the shaved area on the opposite side. At varying intervals after these applications the animals were killed by decapitation, the skin removed, separated from subcutaneous tissue, and sliced. The oxygen uptake of the slices from the normal side was then compared with that of the slices from the side painted with lewisite; by this means the respiration of the contaminated skin was always compared with that of normal skin from the same animal, thus obviating the extensive differences in respiratory rates that exist among the skins of different animals. Pyruvate and succinate were again used as substrates.

Preliminary experiments were first carried out to determine approximately the lowest concentration of lewisite which, when applied to the shaved rat's skin, would produce visible pathological changes; it was found that 0·2 ml. of 0·1% solution of lewisite in ethanol, when spread over the shaved area produced a visible degree of oedema within 30 min. In the rat, under the conditions of these experiments, oedema of the skin and subcutaneous tissues was the first sign of damage; erythema did not usually occur until later, and was then only faint. Vesication was never seen.

The results of these experiments are summarized in Table 3. In column 1 of Table 3 an approximate estimate of the amount of lewisite applied to unit area of skin is given, calculated from the total weight of skin used. Column 2 shows the time that elapsed between the application of the lewisite and the killing of the animal. It will be seen that if the animal is killed 5 min. after the application of these concentrations of lewisite to the skin there is already a marked inhibition of pyruvate oxidation but no apparent inhibition of succinate oxidation (when calculated as extra O_2 uptake due to the added succinate). With these concentrations of lewisite there are no visible changes in the skin at the end of 5 min. It must be pointed out, however, that although the skin was removed from the

Table 3. *Comparison of the oxidation of pyruvate and succinate by normal skin and by skin contaminated with lewisite*

(Results as μl. O_2/g./hr.)

Approx. amount lewisite (μg./g. skin)	Duration of action (min.)	Residual		Pyruvate		Succinate	
		Normal	Lewisite	Normal	Lewisite	Normal	Lewisite
45	40	190	135	212	148	278	—
60	50	159	87	209	113	388	289
70	5	176	99	217	90	272	264
90	45	195	100	256	134	327	269
95	30	264	136	286	95	346	301
125	25	213	71	246	70	311	178
140	30	235	45	229	44	369	158
150	5	141	87	154	87	235	226
150	5	144	97	172	104	258	218
160	35	127	50	157	42	237	134

Extra respiration in above experiments (i.e. substrate-residual)

Pyruvate		Succinate		Inhibition (%)	
Normal	Lewisite	Normal	Lewisite	Pyruvate	Succinate
22	13	88	—	41	—
50	26	229	201	47	13
41	0	96	165	100	0
61	34	132	169	44	0
22	0	82	165	100	0
33	0	98	107	100	0
0	0	134	113	—	15
13	0	94	139	100	0
28	7	114	121	75	0
30	0	110	84	100	26

animals 5 min. after contamination actual measurements of oxygen consumption were not begun until a further 30–40 min. had elapsed.

In those experiments in which a longer time was allowed to elapse before killing the animal, visible oedema was present in each case. In these experiments also there was a large inhibition of pyruvate oxidation in the lewisite-contaminated skin, whereas succinate oxidation again was only very slightly inhibited or, in some cases, actually stimulated.

If the oedema were responsible for an apparent inhibition of pyruvate oxidation owing to 'dilution' of the enzyme systems, it would be expected that succinate oxidation would be inhibited to the same degree. Although this was not found to be so, in two experiments portions of skin from the normal side and from the side painted with lewisite were dried at 110°; in these two cases the lewisite-contaminated skin contained only 8 and 2 % more water respectively. Moreover, when the skin is removed from the body it is apparent that most of the fluid is present in the subcutaneous tissue. The oedema may therefore be safely excluded as contributing to this diminished respiratory rate by any dilution effect. Concentrations of lewisite of 1 % and higher were found to inhibit both pyruvate and succinate oxidation.

DISCUSSION

The experiments described above indicate that a concentration of lewisite sufficient to produce very considerable oedema when applied to the skin of rats regularly produces a marked fall in the rate of respiration of the contaminated skin. An attempt has been made to analyze this inhibition of respiration in terms of specific oxidations. The interpretation of this aspect of the work is rendered difficult on account of the small and very variable oxygen uptake induced by the presence of added pyruvate. Using 2–5-day-old rat skin Needham & Dixon (1941) have indeed stated that the addition of either glucose or pyruvate failed to cause any effect on oxygen uptake. In the present work, in a very few experiments, as already pointed out, no extra respiration was observed, but in every case where the addition of pyruvate did result in increased respiration, there has been a substantial decrease, amounting to 40–100 %, in the extra uptake of the contaminated skin. This is in striking contrast to the extra respiration induced by the presence of succinate, the oxidation of which is only very slightly inhibited by these concentrations, and is in fact more frequently apparently stimulated. This finding with succinate indicated, therefore, that the damaging action of lewisite on skin is not due to a

generalized denaturant or coagulant effect on the skin proteins. Although the evidence presented above is insufficient to warrant a firm conclusion that vesication is initiated by this respiratory defect, yet, since pyruvate oxidation is known to be a fundamental stage in the energy-yielding reactions of many animal cells, it is felt that inactivation of carbohydrate metabolism at this stage may represent a biochemical lesion sufficiently severe to account for grave alterations in the functions of the capillary endothelial cells. It should be pointed out that the defect in pyruvate metabolism is certainly present at an early stage in the development of the skin lesion, although no evidence has as yet been obtained that it actually precedes the development of visible oedema, since, by the time respiratory measurements were actually begun, using the skin removed 5 min. after contamination, it might be argued that oedema would also have been present had the skin still been on the animal.

SUMMARY

1. The effect of low concentrations of sodium arsenite and lewisite on the respiration of rat skin slices in the presence of pyruvate and succinate has been studied.

2. Under the conditions used, addition of these arsenicals caused a marked inhibition of the small extra oxygen consumption due to pyruvate (86–100 %), but only a slight effect on succinate oxidation (0–19 %).

3. The respiration of skin contaminated *in vivo* with lewisite has been compared with that of normal skin slices. In the contaminated skin there was again a marked inhibition of pyruvate oxidation but no significant inhibition of succinate oxidation.

4. The significance of these changes to the pathogenesis of vesication is briefly discussed.

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British Anti-Lewisite

1. ARSENIC DERIVATIVES OF THIOL PROTEINS

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The basic mechanism by which arsenic injures living cells and exerts its toxic effect on the whole organism, whether protozoal or metazoal, has been for many years the subject of extensive research. To a large degree this has been the outcome of the introduction of organic compounds of arsenic for chemotherapeutic purposes. Subsequently arsenic was introduced for offensive purposes against man, in the form of the arsenical smokes and vesicants, once more focussing interest on the problem of its mode of action and on the prevention or treatment of its effects.

Although arsenic has in the past been described as a 'general protoplasmic poison' it is not an