CXXXVI. THE EFFECT OF ARSENIC UPON SOME OXIDATION-REDUCTION SYSTEMS.

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IN 1887 Jonathan Hutchinson pointed out that persons who took arsenic for long periods were liable to cancer of the skin. At the present time records of about forty cases of this form of cancer of the skin can be collected from the literature [Kennaway, 1925]: in a large proportion of these the arsenic was taken in the form of potassium arsenite (Fowler's solution). Now, cancer can be produced experimentally by the action of another class of chemical compounds, namely, certain constituents, as yet unidentified, of coal-tar [Bayon, 1912], shale oil, and some petroleums [Leitch, 1922, 2, 1924]. The evidence available suggests that the active compounds in these complex mixtures are (1) of high boiling point, (2) neutral, (3) free from nitrogen and sulphur, and it seems probable that they are hydrocarbons of high molecular weight [Bloch, 1922; Kennaway, 1924]. Hence cancer of the skin can be induced by two wholly different chemical agents, namely, by arsenites and by high-boiling hydrocarbons, which seem to have no characters in common. The experiments described below represent an attempt to investigate this anomaly.

In a case in which arsenic cancer develops there is usually a prolonged period of hyperkeratosis of the palms and soles before any malignant change occurs. As an actual instance one may take that recorded by Semon [1922]. The patient took arsenic from 1894 to 1901: in the latter year hyperkeratosis was present and this persisted without further intake of arsenic until the date of observation (1922). In any attempt to investigate the part played by arsenic in such a case one requires to have some idea of the concentration of it in the skin during the precancerous stage. There is in the literature a very large number of data on the quantities of arsenic found (1) in the hair of persons who had taken known amounts of Fowler's solution over short periods, and (2) in the hair of rats and the feathers of fowls to which arsenic had been administered [Report of Royal Commission, 1903]. We have found only one set of analyses on the tissues of a human subject who could be considered to be in any stage of the precancerous condition. This was recorded by Dixon Mann [1914] who found, in a case in which there was pronounced hyperkeratosis, that "the true skin contained an unweighable amount, whereas the horny scales yielded equal to 0.013 %, and the hair equal to 0.008 % of arsenious

oxide. The nails probably contained still more, judging from the deposits yielded by the small available weights of nail-clippings."

As we were unable to find any fuller account of the case of hyperkeratosis, these scanty data had to serve as the basis of the experiments described in the present paper. We tested the effect of the concentrations of arsenic found by Dixon Mann (*i.e.* about 10 mg. arsenic in 100 cc.) upon three oxidation-reduction systems, namely:

- (1) hypoxanthine and the xanthine oxidase of rat and mouse skin;
- (2) acetaldehyde and colloidal platinum;
- (3) acetaldehyde or propaldehyde, glycine and phosphate.

Unfortunately we have so far been unable to detect with any regularity the oxidation-reduction changes which must undoubtedly occur in human skin [Kaye, 1924; Walker, 1925]. A large number of experiments upon extracts and suspensions of this tissue, obtained generally from freshly excised breasts, with various combinations of methylene blue, indigo carmine, hypoxanthine, acetaldehyde and sodium succinate have not given reduction which was sufficiently constant to allow the effect of arsenite upon it to be demonstrated. Decoloration was often absent, or very irregular in a series of tubes, or extremely slow. Various causes may contribute to this; human skin as obtained in the ordinary way consists very largely of the inert connective tissue of the dermis and is excessively tough for thorough mincing; and possibly xanthine oxidase, which has a very capricious distribution in different species and tissues [Morgan, 1926], is absent; or the insolubility of the substance in skin giving the nitroprusside reaction [Walker, 1925] may render the technique used in those experiments unsuitable. Further experiments are in progress by which it may be possible to obtain some indication of the oxidations in human skin and of the effect of arsenic upon these. In the meantime we have used the skin of the rat and mouse.

Spitzer [1898] showed that some tissues convert As_2O_3 into As_2O_5 ; that is, the As_2O_3 in this case acts as an oxygen acceptor. Our experiments were begun with the expectation that arsenic trioxide would act thus as an oxygen acceptor under other conditions also, and might bring about reduction in the same manner as do hypoxanthine or aldehyde when in the presence of xanthine oxidase or colloidal platinum; but the results showed at once that arsenite had an inhibitory instead of an accelerating action. It was found most convenient to make the solutions of arsenite and arsenate of such strength that they contained arsenic in about ten times the concentration found by Dixon Mann: if one then added the solution to the mixture in the Thunberg tube in such amounts as to make up one-tenth of the whole, the percentage of arsenic found in the hyperkeratotic tissues was provided. The arsenite solutions used were derived from three sources.

(1) A preparation of sodium arsenite, obtained from chemical manufacturers, stated to be NaAsO_2 . A solution of 0.173 % strength contains 0.1 % As.

(2) In order to obtain a very pure material, As_2O_3 was sublimed and a weighed amount of the sublimate dissolved with the amount of NaOH calculated to give NaAsO_2 (*i.e.* 0.2653 g. As_2O_3 dissolved with 26.6 cc. 0.1 *N* NaOH and made up to 200 cc.; this contains 0.1 % As).

(3) Fowler's solution was used in order to test the action of arsenic in the actual form which has been taken in most cases of arsenic cancer.

Fowler's solution (liquor arsenicalis) consists of

Arsenious oxide	...	...	...	...	10 g.
Potassium carbonate	...	...	...	...	10 g.
Compound tincture of lavender	...	...	...	...	30 cc.
Water to	...	...	...	...	1 l.

It thus contains 1.0 % As_2O_3 and 0.75 % As . This solution contains excess of alkali, the calculated amount of K_2CO_3 to form KAsO_2 being 7 g.

The arsenate solution used contained 0.25 % Na_2HAsO_4 , obtained from chemical manufacturers; this is equivalent to 0.1 % As .

XANTHINE OXIDASE OF RAT AND MOUSE SKIN.

A large number of preliminary tests were required in order (1) to decide upon a technique for obtaining sterile extracts of skin; filtration through a candle was found to give inactive fluid; (2) to ascertain the optimum proportions of hypoxanthine and methylene blue; too great an excess of the former inhibits the reaction [see Dixon and Thurlow, 1924].

The following method was finally adopted. (The phosphate solution referred to throughout this paper was Sørensen's mixture of p_{H} 7.5.)

(1) The extracts of skin were prepared by several different methods.
 (a) A rat or several mice were killed, the bodies epilated with Na_2S and washed in running water. The skin was removed, minced and placed in phosphate solution (1 cc. to 1 g.) either saturated with toluene, or containing 0.1 % acriflavine. The mixture was shaken for 15 minutes, and after this stage was brought into contact with sterile materials only. It was strained through cotton wool or linen and centrifuged, and the supernatant fluid was kept in the ice-chest overnight in the presence of toluene when flavine was not used.
 (b) The removal of hair at the start of course assists greatly in sterilisation, but the smell of sulphide was found to be perceptible in epilated skin even after very prolonged washing, and it was feared that the compound might bring about reduction. Hence in some cases the body was soaked in .5 % lysol, washed in water for an hour and the unepilated skin minced and treated further as stated above.

(2) To avoid sterilisation by heat with possible decomposition, 10 mg. hypoxanthine were dissolved in 0.75 cc. 0.1 *N* NaOH and 9.25 cc. boiled phosphate solution by warming in a sterile tube; a few drops of toluene were added, the whole was shaken and allowed to stand for some hours before use. It was found very useful, when all the materials for an experiment were ready, to make a rough preliminary aerobic test with amounts of hypoxanthine in the

ratio 1 : 2 : 3 : 4, in order to ascertain the optimum, which varies with different skins.

(3) The methylene blue (1 in 5000 in phosphate solution) and arsenic solutions were placed first in the Thunberg tubes which were then wool-plugged and autoclaved, the taps being sterilised separately and touched with sterile vaseline before insertion. The skin extract, hypoxanthine, and two drops of toluene were then added and the tubes evacuated with a Fleuss pump. The side tubes were wool-plugged and the taps not turned on until the pressure had been lowered, so that no contamination by entry of air could occur. The tubes were in some cases simply evacuated, in others refilled twice with nitrogen, and were then incubated at 40°.

Details of five experiments with skin are given in Tables I–III. The results show that arsenic in the concentrations found by Dixon Mann (about 10 mg. in 100 cc.) prolong the reduction time five to tenfold, while smaller amounts (2 to 7.5 mg. in 100 cc.) cause slowing to two to five times the control time. Although the delay with the larger amounts of arsenic is obvious, it is difficult to record this in exact figures, because the last stages of the reduction are so drawn out that it is not easy to decide when the last trace of blue disappears, and often the process comes to a standstill when almost complete (see for instance Table III). No doubt it would be better to compare the times of half-reduction.

Arsenate (equivalent to 10 mg. As in 100 cc.) was tested in four experiments only; in three of these the reduction time was the same as in the control (Table II, Extract B); in the fourth there was a slight retardation (from 8 to 11 mins.; Table II, Extract A).

Table I.

Tube	Rat skin extract (cc.)	Methylene blue 1 in 5000 (cc.)	Hypo- xanthine 1 mg. in 1 cc. (cc.)	NaAsO ₂ from sublimed As ₂ O ₃ (cc.)	Phosphate solution (cc.)	Reduction time (mins.)	Approx. conc. of arsenic (mg. in 100 cc.)
1	0.4	0.45	0.1	—	1.0	c. 6	—
2	0.4	0.45	0.1	—	1.0	c. 6	—
3	0.4	0.45	—	—	1.0	? begun 60	—
4	0.4	0.45	0.1	0.2	0.8	c. 42	10
5	0.4	0.45	0.1	0.2	0.8	c. 60	10
6	0.4	0.45	0.1	0.1	0.9	a.c. 19 then very slow	5
7	0.4	0.45	0.1	0.1	0.9	Ditto	5
8	0.4	0.45	0.1	0.07	1.0	c. 14	3.5
9	0.4	0.45	0.1	0.07	1.0	c. 16	3.5
10	0.4	0.45	0.1	0.04	1.0	c. 7	2
11	0.4	0.45	0.1	0.04	1.0	c. 14	2

Cultures in broth from all tubes were sterile.

The ratio of hypoxanthine to methylene blue is 3 mols. : 1 mol.

In this and subsequent tables c. = complete; a.c. = almost complete.

The possibility must be considered that the arsenical solutions act thus in virtue of their alkalinity: but (1) the addition of the minute amount of alkali to the large volume of buffer produces no change detectable by neutral

Table II.

Tube	Rat skin extract (cc.)	Methylene blue 1 in 5000 (cc.)	Hypo- xanthine 1 mg. in 1 cc. (cc.)	NaAsO ₂ 0.173 % (cc.)	Na ₂ HAsO ₄ 0.25 % (cc.)	Phos- phate solution (cc.)	Reduction time (mins.)	Approx. conc. of arsenic (mg. in 100 cc.)
Extract A:								
1, 2	0.5	0.45	0.07	—	—	1.0	c. 8, 8	—
3, 4	0.5	0.45	—	—	—	1.0	Nil 60	—
5, 6	0.5	0.45	0.07	0.2	—	0.8	a.c. 60	10
7, 8	0.5	0.45	0.07	0.4	—	0.6	Advanced 60	20
9, 10	0.5	0.45	0.07	—	0.2	0.8	c. 11, 11	10
11, 12	0.5	0.45	0.07	—	0.4	0.6	c. 11, 11	20
Extract B:								
1, 2	0.5	0.45	0.04	—	—	1.0	a.c. 89	—
3	0.5	0.45	—	—	—	1.0	Nil 89	—
NaAsO ₂ from sublimed As ₂ O ₃								
4, 5	0.5	0.45	0.04	0.2	—	0.8	Nil 89	10
6, 7	0.5	0.45	0.04	0.1	—	0.9	Nil 89	5
Fowler's solution 1/10								
8, 9	0.5	0.45	0.04	0.2	—	0.8	Nil 89	10
10, 11	0.5	0.45	0.04	—	0.2	0.8	a.c. 89	10

Cultures in broth from all tubes in series A were sterile; tubes in series B were not tested.

The ratio of hypoxanthine to methylene blue is, with Extract A, 2.1 mols. : 1 mol., and with Extract B, 1.2 mol. : 1 mol.

Table III.

Tube	Mouse skin extract (cc.)	Methylene blue 1 in 5000 (cc.)	Hypo- xanthine 1 mg. in 1 cc. (cc.)	Fowler's solution 1 in 10 of phosphate solution (cc.)	Phos- phate solution (cc.)	Reduction time (mins.)	Approx. conc. of arsenic (mg. in 100 cc.)
Extract A (3 skins):							
1	0.5	0.45	0.15	—	1.0	25	—
2	0.5	0.45	0.15	—	1.0	28	—
3	0.5	0.45	0.15	0.2	0.8	70	7.5
4	0.5	0.45	0.15	0.2	0.8	85	7.5
Extract B (3 skins):							
NaAsO ₂ 0.173 %							
1	0.4	0.45	0.15	—	1.0	22	—
2	0.4	0.45	0.15	—	1.0	20	—
3	0.4	0.45	—	—	1.0	Nil in 90	—
4	0.4	0.45	0.15	0.1	0.9	a.c. 49	5
5	0.4	0.45	0.15	0.1	0.9	a.c. 90	5
6	0.4	0.45	0.15	0.1	0.9	77	5
7	0.4	0.45	0.15	0.07	1.0	43-49	3.5
8	0.4	0.45	0.15	0.07	1.0	43	3.5
9	0.4	0.45	0.15	0.07	1.0	49	3.5
10	0.4	0.45	0.15	0.04	1.0	43-49	2
11	0.4	0.45	0.15	0.04	1.0	a.c. 90	2
12	0.4	0.45	0.15	0.04	1.0	43	2

The ratio of hypoxanthine to methylene blue is 4.5 mols. : 1 mol.

Last stages of reduction in B tubes 4-12 were very slow and irregular, hence exact times of reduction were difficult to fix. Contents of all B tubes showed same tint on addition of neutral red.

red (Table III); (2) in earlier experiments in which the arsenical solutions were neutralised first of all the same changes were observed; (3) xanthine oxidase is not sensitive to changes of p_H from 5.8 to 9.0 [Dixon and Thurlow, 1924]; (4) with colloidal platinum, retardations of the same type are produced by neutralised arsenical solutions in the presence of 0.01 *N* NaOH.

COLLOIDAL PLATINUM.

Bredig and Sommer [1910] showed that platinum and other metals in the colloidal state could bring about the reduction of methylene blue by aldehydes in an alkaline medium. Their colloidal solutions were made by electrical dispersion. In the experiments recorded here, preparations¹ made by reduction were used. Acetaldehyde was taken throughout as oxygen acceptor; hypoxanthine does not act in conjunction with platinum. The alkaline reaction required was produced by adding to the mixture in the Thunberg tube one-tenth of its volume of 0.1 *N* NaOH. The ratio of acetaldehyde (0.33 or 1.0 cc. of 2 vols. % solution) to methylene blue (4 cc. of 1 in 5000) was from 56 to 169 times the theoretical equimolecular proportion; in the experiments of Bredig and Sommer this ratio is greater still, being 1000 : 1 or more.

Table IV.

Tube	Colloidal Pt 0.1 % (cc.)	Neutral CH ₃ CHO 2 vols. % (cc.)	Methylene blue 1 in 5000 (cc.)	NaOH 0.1 <i>N</i> (cc.)	H ₂ O (cc.)	Neutral NaAsO ₂ 0.173 % = 0.1 % As (cc.)	Neutral Na ₂ HAsO ₄ 0.25 % = 0.1 % As (cc.)	Re- duction time (mins.)
1	1.0	1.0	4.0	0.7	0.7	—	—	6
2	1.0	1.0	4.0	0.7	0.7	—	—	5
3	1.0	1.0	4.0	0.7	0.7	—	—	6
4	1.0	1.0	4.0	0.7	—	0.7	—	267
5	1.0	1.0	4.0	0.7	—	0.7	—	a.c. 267
6	1.0	1.0	4.0	0.7	—	0.7	—	a.c. 267
7	1.0	1.0	4.0	0.7	—	—	0.7	7
8	1.0	1.0	4.0	0.7	—	—	0.7	8
9	1.0	1.0	4.0	0.7	—	—	0.7	7

Tubes evacuated, filled with nitrogen, and incubated at 40°.
For concentrations of arsenic see Table V.

In all, 18 experiments were carried out with colloidal platinum. Of 15 experiments with arsenite, 13 gave retardation of the type summarised in Table V; of the remaining two, in which the ratio of methylene blue to platinum was high, one gave very slow and irregular results, and in the other, the arsenic in the lower concentrations (1.0 and 0.1 mg. per 100 cc.) caused acceleration, for which no explanation could be found. A detailed record of an experiment is given in Table IV, and a summary of a number of results in Table V.

The results given in Table V show that arsenic, as arsenite, when in concentrations of the order observed by Dixon Mann (*e.g.* 9.5 mg. in 100 cc.) retards the reduction of methylene blue by platinum and aldehyde by as much as twenty- or forty-fold; but when the amount of arsenic is reduced to

¹ Obtained from the Crookes Laboratories, London.

one-tenth of this (*i.e.* to 0.95 mg. in 100 cc.) the reduction time is prolonged not more than $2\frac{1}{2}$ times; and at still lower concentration (0.095 mg. in 100 cc.) the retardation is slight or absent, and in one case there was even an acceleration (to 0.85 of the control time). Under the conditions adopted, the platinum-aldehyde system is more affected by a given concentration of arsenite than is the xanthine oxidase of rat and mouse skin. If the reaction be slowed by increasing the ratio of methylene blue solution to those of platinum and aldehyde from 4 : 1 to 12 : 1 the general character of the results is not altered.

Table V.

Colloidal platinum and arsenite.				
CH ₃ CHO Pt	Ratio Methylene blue	Arsenic in 100 cc. in whole mixture (mg.)	Mean reduction time in controls (mins.)	Mean reduction time in presence of arsenic if control reduction time = 1
	1 : 4	4.3	5.5	42.0
	1 : 4	9.5	5.6	47.0
	1 : 12	9.5	Less than 1350	(Partial in 1350)
	1 : 4	9.5		5.0
	{	0.95	82.0	1.06
		0.095		1.07
		0.95	5.0	1.4
		0.095		1.0
	{	9.5	67.5	20.4
		0.95		1.01
		0.095		1.05
		9.5	6.0	36.0
	0.95	2.08		
	0.095	1.1		
	{	0.98	71.0	2.3
		0.098		0.85
CH ₃ CHO : Pt : MB		4.3	23.5	58.0
	1 : 4 : 16			

The figures in the first column indicate the relative volumes of the following solutions:

CH₃CHO = 2 vols. % CH₃CHO in water neutralised with NaOH.
Pt = 0.1 % colloidal platinum.
MB = 1 in 5000 methylene blue in water.

In all the experiments recorded except the last the volumes of aldehyde and platinum solutions were equal.

Arsenite solution used was neutralised 0.173 % NaAsO₂ (= 0.1 % As).

Tubes were evacuated, filled with nitrogen and incubated at 40°.

Thus the platinum-aldehyde system seems to be especially sensitive to concentrations of arsenic of an order not less than those observed in hyperkeratotic skin. The lower percentages tested (0.95 to 0.095 mg. per 100 cc.) are similar to those found in the hair of persons who have been taking medicinal doses of Fowler's solution for 1 or 2 months (2.0 to 0.16 mg. per cent.) [Report of Royal Commission, 1903].

Arsenate (equivalent to 10 mg. As in 100 cc.) was tested in five experiments of which one gave very discordant results in duplicate tubes, and was rejected. The remaining four experiments gave mean reduction times of 12.2' in the controls and 16.3' in the presence of arsenate, *i.e.* a retardation of 25 % only.

THE GLYCINE-ALDEHYDE-PHOSPHATE SYSTEM.

A series of experiments was made also with the glycine-phosphate-aldehyde system of Haehn and Pülz [1924]. The action of this is illustrated by the experiments recorded in Table VI which shows (Exp. 1) that the omission of any one of the three constituents renders the course of the reaction extremely slow, or stops it altogether. Exp. 11 shows further that increase of phosphate does not compensate in any way for absence of the amino-acid. The results obtained with the Haehn system have been irregular: the identity of the reduction times in triplicate tubes seen in Exp. 11 (Table VI) is exceptional. More often there is a fairly wide range of variation, and we adopted the method of putting up 3, 4 or 5 identical tubes of each mixture in order to eliminate

Table VI.

Tubes <i>Exp. 1</i>	H ₂ O (cc.)	Glycine 0.125 <i>M</i> in H ₂ O (cc.)	Phosphate (cc.)	CH ₃ CHO 2 vols. % in H ₂ O (cc.)	Methylene blue, 1 in 5000 in H ₂ O (cc.)	Reduction time
1	—	2.5	2.5	0.1	0.25	c. 30'
2	—	—	5.0	0.1	0.25	a.c. next day
3	2.5	2.5	—	0.1	0.25	Partial next day
4	—	2.5	2.5	—	—	Partial next day
<i>Exp. 11</i>		Glycine 0.125 <i>M</i> in phosphate		CH ₃ CHO 0.2 vols. % in phosphate	Methylene blue, 1 in 5000 in phosphate	NaAsO ₂ neutral 0.173 %
1, 2, 3	0.5	2.5	—	1.0	0.25	—
4, 5, 6	—	2.5	—	1.0	0.25	0.5
7, 8, 9	0.5	—	2.5	1.0	0.25	—
10, 11, 12	—	—	2.5	1.0	0.25	0.5
						c. 20, 20, 20'
						c. 25, 25, 25'
						Nil in 24 hours
						Nil in 24 hours

Phosphate = Sørensen's solution of p_H 7.5. Tubes evacuated, filled with N₂, and incubated at 40°. Tubes 4–6 and 10–12 in Exp. 11 contain 0.012 % As.

Table VII.

	H ₂ O (cc.)	Glycine 0.125 <i>M</i> (cc.)	CH ₃ CHO 0.2 vols. % (cc.)	Methylene blue, 1 in 5000 (cc.)	NaAsO ₂ neutralised 0.173 % (cc.)	Reduction time (mins.)
<i>Exp. 6</i>	0.5	2.5	2.0	0.25	—	45, 45, 48, 45, 45 mean 45.6
6	—	2.5	2.0	0.25	0.5	57, 53, 57, 59, 61 mean 57.4
<i>Exp. 7</i>	0.5	2.5	1.0	0.5	—	37, 37, 40, 40, 40 mean 38.8
7	—	2.5	1.0	0.5	0.5	43, 40, 43, 43, 46 mean 43.0

Glycine, CH₃CHO and methylene blue were dissolved in phosphate of p_H 7.5. Tubes evacuated, filled with nitrogen and incubated at 40°.

these irregularities as far as possible by averaging. Two typical examples of such results are given in Table VII. Using multiple tubes, 13 experiments in all were made: of these, three were rejected as the results in identical tubes were too divergent to be averaged, and in one of these three the arsenic seemed to accelerate reduction considerably. The remaining ten experiments are summarised in Table VIII. These show that arsenite in

amounts corresponding to the concentrations of arsenic observed by Dixon Mann, or in two or three times that amount, prolongs the reduction time by 18 % on the average. In Exp. 2 (see Table VIII) a retardation of no less than seven-fold was obtained in ten tubes giving concordant readings, but we were unable to repeat this remarkable result which is therefore excluded from the average. The system is evidently very sensitive to small differences in conditions, and this perhaps makes its biological interest all the greater.

Table VIII.

	Mean reduction times (mins.)				Arsenic (mg. in 100 cc.)	
	Control	Sodium arsenite	Control	Sodium arsenate		
Exp. 2	24.0	165.0	—	—	7.4	Propaldehyde
3	28.4	36.4	—	—	7.4	"
4	—	—	54.4	50.6	7.4	"
6	45.6	57.4	—	—	9.5	"
7	38.8	43.0	—	—	11.0	Acetaldehyde
8	67.6	81.0	—	—	20.0	"
9	36.0	40.0	—	—	12.0	"
11	20.0	25.0	—	—	12.0	"
12	23.6	25.6	—	23.0	12.0	"
13	28.6	31.6	—	28.0	11.0	"
Mean omitting Exp. 2	36.1	42.5	35.5	33.8		
Ratio	100	118	100	95		

In all the later experiments the tubes were placed in a dark room during incubation, on account of the possibility that unequal illumination caused irregularities [cf. xanthine oxidase, Bernheim and Dixon, 1928]; but neither this change, nor the substitution of acetaldehyde for propaldehyde, made the results distinctly more constant.

With arsenate three experiments only were carried out; these showed a slight acceleration, amounting to 5 % of the control time, and the system therefore differs in this respect from those containing xanthine oxidase or platinum. But in this system arsenate in sufficient concentration (about 0.05 *M*) can take the place of phosphate [Haehn and Pülz, 1924, p. 90].

DISCUSSION.

It is of course obvious that one cannot with any certainty compare reactions which take place in a test-tube in an hour or two with changes which in the human skin require many months or years. But the retarding action of arsenite upon some oxidation-reduction systems *in vitro* perhaps justifies the suggestion that arsenic may induce cancer, not by any direct action, but by causing the accumulation in the tissues of some organic compounds which would otherwise be oxidised or reduced to other forms. This supposition would require that the arsenic in the skin should be in the active form of arsenite, whereas we do not actually know whether it is in this form or in that of the inactive arsenate.

In a case of arsenic cancer such as that described above, in which the hyperkeratotic precancerous stage lasted 13 years or more, it is probable that

all the arsenic has disappeared from the tissues long before malignancy develops. There is of course nothing in these experiments, nor so far as we know in any others, to show what is happening in the tissues during this interval. In the case of tar cancer, the period that can elapse between the cessation of action of the first carcinogenic agent and the development of a tumour has been demonstrated experimentally [Leitch, 1922, 1]. The same process is seen when a cancer of the skin in a mule-spinner, or of the bladder in a dye-worker, appears long after exposure to the harmful agent has ceased.

These experiments provide another illustration of the difference in biological action between arsenite and arsenate. In this laboratory a cancer of the skin was produced in four months in one mouse out of a series of one hundred by painting with a solution of potassium arsenite containing 0.09 % As [Leitch and Kennaway, 1922]. A comparable experiment with an arsenate solution has lasted at the present time for 14 months without the appearance of any tumours; but of course many more positive results would be required to enable one to draw any conclusion from such parallel experiments. It is uncertain whether the skin of the rat, which furnished many of the solutions of xanthine oxidase used, is susceptible to arsenic cancer. In the only case in which tar cancer has been induced in a rat [Herly, 1926], 1 % of As_2O_3 was added to the mixture, but controls with the arsenical solution alone were negative.

SUMMARY.

Arsenic in the form of arsenite, when in concentrations similar to those found in the skin in arsenical hyperkeratosis, has a retarding action upon three oxidation-reduction systems, namely (1) hypoxanthine and the xanthine oxidase of rat or mouse skin; (2) acetaldehyde and colloidal platinum; and (3) propaldehyde or acetaldehyde, glycine and phosphate. Arsenates are comparatively inactive. The possible significance of these findings in connection with the nature of arsenic cancer is discussed.

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