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The Reaction of Nucleic Acids with Mustard Gas

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The delayed healing of lesions caused by mustard gas (*H*) provoked speculation that the cell nuclei are especially concerned, and confirmation of this view has accumulated from various sources. Auerbach & Robson (1942, 1946) showed that the exposure of *Drosophila melanogaster* to *H* in sublethal doses caused reduced fertility and induced visible mutations in succeeding generations. Robson (1943) observed that pollen grains of *Tradescantia* after treatment with *H*, showed widespread disorganization of chromosomes, and Fell & Allsopp (1943) noted similar behaviour in the cells of *H*-treated mouse skin. Koller (1943) records that during the 48 hr. following a short (30 sec.) exposure of *Tradescantia* pollen grains to concentrated *H* vapour, chromosome breakage, structural changes including new rearrangements, and adherences of adjacent chromosomes were induced at various loci in the chromosomes. He observed that the separation of the chromosomes at anaphase and telophase of mitosis was prevented by these attachments. These results focused attention on the action of *H* on nucleoproteins, and Berenblum (1940 *a*, *b*) and Berenblum & Schoental (1947) found that aqueous extracts of skin or solutions of thymus nucleoprotein underwent irreversible precipitation when treated with *H*, the precipitate having a higher sulphur content than the original nucleoprotein.

The action of *H* on proteins has been studied by many investigators, e.g. Berenblum & Wormall (1939); for reviews see Rydon (1943 *a*, *b*). The purpose of the present investigation was therefore to investigate the reaction of *H* with nucleic acids and their components.

EXPERIMENTAL

Choice of nucleic acids for investigation. The ribose nucleic acid of yeast (YNA) and the deoxypentose nucleic acid of calf thymus gland (TNA) were employed in this investigation because of their availability, and because more is known of their structures than of those of nucleic acids from other sources.

Preparation of materials. The samples of yeast ribonucleic acid were obtained commercially and were deproteinized by a combination of the method described by Sevag, Lackmann & Smolens (1938) and the purification process employed by Fletcher, Gulland, Jordan & Dibben (1944). Yeast ribonucleic acid (20 g.), stirred vigorously into water (250 ml.), was brought into solution at pH 7 by the addition of N-NaOH (about 48 ml.). This solution was shaken vigorously with the following volumes of a mixture (4 : 1) of chloroform and amyl alcohol: 200 ml. four times, 100 ml. ten times. After each shaking, the mixture was centrifuged and the aqueous layer containing the nucleic acid was separated from the chloroform layer which contained the protein as a stable gel. When gel formation no longer occurred after shaking, the solution of protein-free nucleic acid was slowly added to a stirred mixture of ethanol (1180 ml.) and concentrated HCl (6·6 ml.). The precipitated acid (12 g.) was centrifuged and washed three times with 66% aqueous ethanol when it was found to be free from chloride ions; it was then washed successively, once with 80 and 100% ethanol, finally with ether, and dried in a vacuum over P_2O_5-KOH at room temperature. Analyses of various samples are recorded in Table 2.

Deaminated yeast ribonucleic acid (DYNA) was prepared from YNA as described by Fletcher, Gulland, Jordan & Dibben (1944). The samples of the sodium salt of deoxypentose nucleic acid of low molecular weight were obtained commercially and deproteinized by the procedure described above. The sample of high molecular weight had

been prepared from calf thymus glands by Gulland, Jordan & Threlfall (1947). Analyses are given in Table 2. Guanylic acid in the form of its sodium salt was prepared by alkaline hydrolysis of YNA. The pure *H* used was provided by the Chemical Defence Research Department, Porton.

paper. (Before these tests were made, the samples were thoroughly washed with ethanol and ether.) The absence of free *H* in the products was confirmed by the consistent failure to detect the presence of chlorine as a component by means of qualitative tests.

Table 1. *Conditions of reaction of H with nucleic acids*

Reference no. of nucleic acid	Wt. of nucleic acid (g.)	Wt. of *H* (g.)	Mol. of *H*/tetra-nucleotide	Vol. of solution (ml.)	Time of stirring (hr.)	Reference no. of A product	Yield of A product (g.)	Reference no. of B product	Yield of B product (g.)	Yield of A / Yield of B
YNA:										
Y3/20	1·50	0·75	4·1	8·0	10·0	HY3/22A	1·00	HY3/22B	0·55	1·82
Y4/26	4·00	5·00	10·2	30·0	6·0	HY4/30A	3·60	HY4/30B	0·30	12·00
Y5/42	3·00	1·90	5·1	20·0	10·0	HY5/45A	1·40	HY5/45B	0·90	1·56
Y5/42	3·00	1·90	5·1	40·0	10·0	HY6/46A	1·15	HY6/46B	0·85	1·35
Y5/42	3·00	1·90	5·1	60·0	10·0	HY7/47A	1·50	HY7/47B	0·75	2·00
Y6/48	3·00	1·90	5·1	20·0	3·0	HY8/49A	0·10	HY8/49B	3·05	0·03
Y6/48	3·00	1·90	5·1	20·0	6·0	HY9/50A	0·50	HY9/50B	2·55	0·20
DYNA:										
DY2	0·50	0·25	4·1	8·0	22·0	HDY1/25A	0·15	HDY1/25B	0·25	0·60
DY8	6·80	8·00	9·6	48·0	7·0	HDY2/34A	2·18	HDY2/34B	4·10	0·53
HDY2/34B	2·00	3·00	13·7	20·0	10·0	HHDY3/43A	1·00	HHDY3/43B	0·10	10·00
TNA:										
T1/19*	0·75	0·70	7·3	8·0	10·0	HT1/27A	0·75	HT1/27B	0·02	37·5
T2/31*	1·50	1·50	7·3	23·0	11·0	HT2/36A	1·15	HT2/36B	0·35	3·3
T5†	2·00	1·30	5·8	450·0	9·0	HT3/53A	0·01	HT3/53B	1·60	0·006

YNA = yeast nucleic acid; DYNA = deaminated yeast nucleic acid; TNA = thymonucleic acid.
* Degraded low mol. wt. samples. † High mol. wt. sample (tetrasodium salt).

Table 2. *Analytical data for H-treated nucleic acids*

Nucleic acid			A product					B product			
Reference no.	N (%)	P (%)	Reference no.	S (%)	N (%)	P (%)	Na (%)	Reference no.	S (%)	N (%)	P (%)
YNA:											
Y3/20	16·7	9·1	HY3/22A	4·4	14·3	7·6	—	HY3/22B	4·3	14·9	8·2
Y4/26	16·5	9·4	HY4/30A	3·1	14·5	8·1	3·2	HY4/30B	2·8	14·4	8·6
Y5/42	15·9	9·2	HY5/45A	3·5	13·6	7·7	—	HY5/45B	3·8	13·5	8·3
Y5/42	15·9	9·2	HY6/46A	4·3	13·2	7·5	—	HY6/46B	4·7	13·5	8·2
Y5/42	15·9	9·2	HY7/47A	4·9	13·4	7·6	—	HY7/47B	5·2	13·3	7·9
Y6/48	15·6	9·2	HY8/49A	1·6	14·0	7·7	—	HY8/49B	1·5	15·0	8·8
Y6/48	15·6	9·2	HY9/50A	3·0	14·0	7·6	—	HY9/50B	2·8	14·7	8·4
DYNA:											
DY2	11·7	7·6	HDY1/25A	5·0	—	—	—	HDY1/25B	5·1	11·2	7·4
DY8	—	—	HDY2/34A	4·3	10·6	7·2	3·4	HDY2/34B	4·6	10·4	7·9
HDY2/34B	10·4	7·9	HHDY3/43A	7·9	9·2	6·7	—	HHDY3/43B	6·7	8·3	7·5
TNA:											
T1/19*	—	—	HT1/27A	3·4	13·9	8·6	1·0	HT1/27B	—	—	—
T2/31*	16·4	9·5	HT2/36A	3·7	13·3	8·2	3·4	HT2/36B	3·5	12·9	8·9
T5†	15·3	9·3	HT3/53A	—	—	—	—	HT3/53B	4·9	13·3	8·6

YNA = yeast nucleic acid; DYNA = deaminated yeast nucleic acid; TNA = thymonucleic acid.
* Degraded low mol. wt. samples. † High mol. wt. sample (tetrasodium salt).

Criteria of reaction of H with nucleic acids. The criteria of positive chemical reaction between *H* and nucleic acids were as follows: (i) the qualitative demonstration of the presence of sulphur in the products, followed later by detailed analytical investigation; (ii) the demonstration of the absence of uncombined *H* in the products by prolonged passage of air from the sample through S.D. (1942) reagent

The reaction of H with nucleic acids. No interaction could be detected between YNA and *H* as vapour or dissolved in liquid paraffin. When, however, both were dissolved in aqueous ethanol at pH 7, a reaction occurred. The solubility of *H* in aqueous ethanol increases with the concentration of alcohol, whereas that of nucleic acids decreases. The highest concentration of ethanol which could be employed without

the precipitation of nucleic acid was 35%, and this concentration was employed throughout the experiments described below; the presence of ethanol, moreover, considerably reduces the hydrolysis of *H* (Mohler & Hartnagel, 1941; Ogston, 1941) which is a serious competing reaction. When the reaction was carried out in aqueous media with *H* in suspension, the reaction between *H* and nucleic acid was found to be almost negligible.

The results of a series of experiments made with different proportions of *H* and YNA are summarized in Tables 1 and 2. Two products, designated A and B respectively, were always formed, but in various proportions depending on the conditions of the experiment. The A compounds separated from the reaction mixture at pH 7 during the course of the experiment, whereas the B compounds were precipitated by the addition of a mixture of HCl and ethanol after removal of the A compounds at the conclusion of the preparation.

The following description is typical of the experiments which were carried out, and illustrates the procedure. Yeast ribonucleic acid (Y 5/42; 3 g.) was suspended in water and brought into solution at pH 7 by the addition of approximately N-NaOH (about 7 ml.), the final volume being 13 ml. Ethanol (7 ml.) was added, then *H* (1·9 g.), and the mixture was stirred mechanically for 10 hr., the reaction being frequently adjusted to pH 7 by the addition of NaOH. The solid, a sodium salt which had separated, was centrifuged, washed several times with dry ethanol, twice with ether, and dried *in vacuo* over P_2O_5-KOH. Yield 1·4 g. (HY 5/45 A).

The supernatant liquid was then added slowly to a stirred mixture of ethanol (110 ml.) and concentrated HCl (0·6 ml.). The precipitated solid was centrifuged, washed with ethanol and then with ether, and dried *in vacuo* as before. Yield 0·95 g. (HY 5/45 B).

The reactions of *H* with DYNA and with TNA were carried out by a similar procedure. The results are summarized in Tables 1 and 2.

The reaction of H with guanylic acid. The method described above for the reaction of *H* with nucleic acids gave only poor yields when applied to the reaction between *H* and guanylic acid and the following modified procedure, which gave a satisfactory yield, was developed. Sodium guanylate (3 g.) was dissolved in hot water (30 ml.), the solution cooled and added to a solution of *H* (11·8 g., 10 mol. ratio) in ether (15 ml.). The mixture was stirred for 72 hr. and kept neutral by periodic additions of NaOH. *H*-guanylic acid was precipitated with ethanol-ether (5 : 1) mixture (450 ml.). The gummy product was centrifuged and washed four times with ethanol and twice with ether. It was redissolved in water (50 ml.) and the *H*-guanylic acid reprecipitated as a solid by the addition of ethanol and ether; a further reprecipitation was necessary to free the product from NaCl. The final separation of the product by centrifuging was exceedingly difficult owing to its colloidal nature. The *H*-guanylic acid (2·1 g.) was dried *in vacuo* over P_2O_5.

Analytical methods. Nitrogen analyses of untreated and *H*-treated materials were carried out by a micro-Kjeldahl technique (Claudatus, 1939) modified by Still (unpublished). Phosphorus contents were determined by a modification (Still, unpublished) of the micro-method of Embden (1921).

Estimations of the sulphur content of *H*-nucleic acids were carried out by the adaptation by Niederl & Niederl (1938) of the Pregl combustion method. The accuracy of this method depends upon the presence of insufficient metal (e.g. sodium) to retain the S present as metallic sulphate. The *H*-nucleic acids conform to this requirement since the P : S ratio is relatively high. After the analysis, the phosphorus remained in the boat as sodium metaphosphate, which was in each case shown qualitatively to be free from sulphate. In some cases, estimations of sulphur were also made by the micro-Carius method; these results confirmed those obtained by the more rapid Pregl method.

The results of analysis for sulphur in *H*-guanylic acid were low and inconsistent by both the Pregl and Carius methods. In the Pregl method, sulphate could be detected in the boat, and the procedure was modified. The residue was extracted with very dilute HCl, the solution filtered and added to the main bulk of sulphate-containing solution; estimations of this sulphate as $BaSO_4$ then gave consistent results.

Sodium analyses were at first carried out by the method of Hoffmann & Osgood (1938). Later, however, a gravimetric method was used in which the sample was wet-ashed with concentrated HNO_3, phosphate removed as barium phosphate, and sodium weighed as Na_2SO_4.

Electrometric titrations were carried out by the method used by Fletcher, Gulland & Jordan (1944), corrections being applied as described by Jordan & Taylor (1946).

Viscosity measurements on *H*-TNA were carried out using Frampton's (1939) modification of the Ostwald viscometer.

RESULTS

Properties of H compounds

The A products from YNA were pale buff, amorphous solids, insoluble in water but dissolving near pH 10. The B products from YNA were cream-coloured solids, soluble in water above pH 3–4, but precipitated in more acid solutions.

The A compounds from DYNA were very similar in behaviour to those from YNA, being insoluble at a pH below 10. The B compounds were soluble in water and were not precipitated at pH 2·5; this solubility in acids is characteristic of DYNA (Fletcher, Gulland, Jordan & Dibben, 1944).

The A and B compounds from TNA resembled their analogues prepared from YNA in most respects, but exhibited at least one important difference in that their solutions were more viscous than those of the original TNA at corresponding concentrations. Aqueous solutions at pH 11–12 of the A compounds prepared from the low molecular weight samples T 1/19 and T 2/31 were far more viscous than the original nucleic acids, but quantitative determinations were not carried out. The viscosities of aqueous solutions of the sample T 5 of high molecular weight and of the B compound (HT 3/53 B) prepared from it are given for various pressures in Fig. 1. Solutions of HT 3/53 B exhibited critical pressures, in magnitude largely dependent on concentration; at pressures below the critical, the solutions formed a gel and did not flow. Critical

pressures have not been determined for T5 (Creeth, Gulland & Jordan, 1947), but if they exist are considerably lower than those of the B compound.

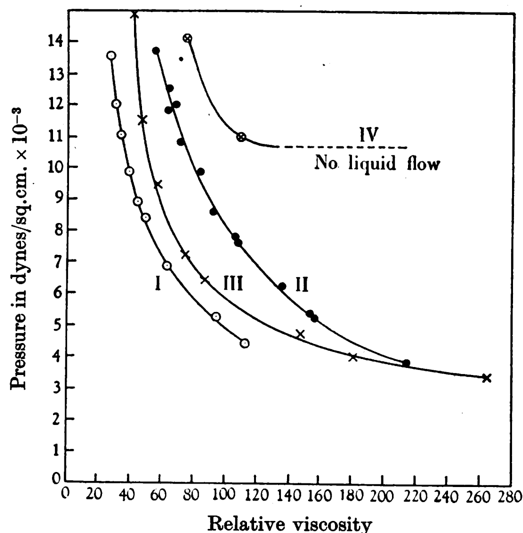


Fig. 1. The viscosities of aqueous solutions of calf thymus deoxypentose nucleic acid (T5) and its H product (HT3/53B). T5: curve I, ⊙, 0·21% solution; curve II, ●, 0·31% solution. HT3/53B: curve III, ×, 0·21% solution; curve IV, ⊗, 0·31% solution.

In air, these H compounds emitted a peculiar odour which was notably increased on treatment with acid and alkali. This odour was reminiscent of the thio-ethers and very similar to that obtained by the reaction of H with ethanolic alkali, known to give divinyl sulphide. In order to investigate the degree of the decomposition of the H compounds caused by the presumed evolution of divinyl sulphide, one sample was analyzed for sulphur immediately after preparation and again after storage for 5 weeks in a loosely corked tube at room temperature. The sulphur contents were 4·35 and 4·30%, respectively. The difference between the two figures is within experimental error and hence no extensive decomposition had occurred.

The identification of groups reacting with H

The electrometric titration of nucleic acids has proved to be a useful method of investigating the structures of nucleic acids, and in particular the number and type of acidic and basic groups present (Fletcher, Gulland & Jordan, 1944; Gulland, Jordan & Taylor, 1947). The titration curves of the H-treated materials have therefore been determined to compare the number of titratable groups present with that of the original nucleic acids. In using this method for the H-nucleic acids, however, it is appreciated that titration may produce fission of the H residues from the nucleic acids, especially in strongly acid and alkaline solutions, and the results obtained may therefore be slightly in error. Throughout, the calculations of numbers of reacting groups are based for convenience on the provisional assumption, sufficiently accurate for present purposes, that the nucleic acids are composed of statistical tetranucleotides (Gulland, Barker & Jordan, 1945; Gulland, 1947).

In order to interpret the data obtained by elementary analysis and by electrometric titration of H compounds, it is necessary to estimate for each sample the formula weight (M) of a statistical H tetranucleotide. This was obtained in each case from the sulphur content, assuming that (i) the number of P atoms/statistical tetranucleotide was the same as in the parent substance, and (ii) the reaction consisted in the replacement of hydrogen atoms by $—CH_2.CH_2.S.CH_2.CH_2.OH$ or $(—CH_2.CH_2)_2S$ groups, the formula weight thus increasing by 104 in the first and 86 in the second case for each sulphur atom introduced.

In the case of substitution by

$$—CH_2.CH_2.S.CH_2.CH_2.OH,$$

if x such residues combine with each statistical tetranucleotide of the parent substance having formula weight m, and if the sulphur content of the product be s% then $s=3200x/(m+104x)$ and hence $x=sm/(3200-104s)$ and $M=m+104x$. Similarly, in the case of substitution by $(—CH_2.CH_2)_2S$ groups, $x=sm/(3200-86s)$ and $M=m+86x$.

The calculation of m presents no difficulty in the case of thymus deoxyribonucleic acid for which it has the value 1235, but in the case of yeast ribonucleic acid and its deamination product, allowance must be made for the deficiency in phosphorus below the theoretical value. Thus, if the percentage of phosphorus be p, m is now the formula weight of the hypothetical tetranucleotide corrected for the deficiency of phosphorus, and δ the atom deficiency of phosphorus/tetranucleotide, then the number of atoms of phosphorus/tetranucleotide is $4-\delta=mp/3100$. Since the loss of a phosphoryl group involves a decrease of 80 in the formula weight of the hypothetical tetranucleotide then $m=1285-80\delta$, and combining with the above equation $m=299{,}150/(310-8p)$.

The calculation of M for all H compounds of nucleic acid is thus possible and, using an analogous method, the formula weight of the H compound of guanylic acid can also be obtained.

The A products of H-TNA, H-YNA and H-DYNA were not sufficiently soluble in aqueous solutions to permit their examination by electrometric titration. The B products, however, were readily soluble, and the results of titrations performed with these compounds and H-guanylic acid are given in Figs. 2, 3, 4 and 5, together with the titration curves of the original substances. It was found to be immaterial whether glass or hydrogen electrodes were used,

since poisoning of the latter did not occur. Table 3 summarizes the number of titratable groups potentially capable of reacting with *H*, the number of such groups remaining unsubstituted after treatment with *H*, and (by difference) the number of groups blocked by *H*. It will be seen that in every case a reaction with *H* has occurred.

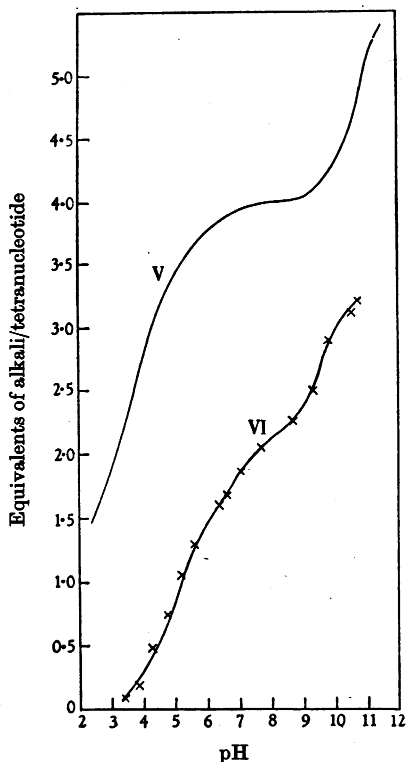


Fig. 2. The electrometric titration of calf thymus deoxypentose nucleic acid (T5), curve V (Gulland, Jordan & Taylor, 1947) and its *H* product (HT3/53B), curve VI, ×.

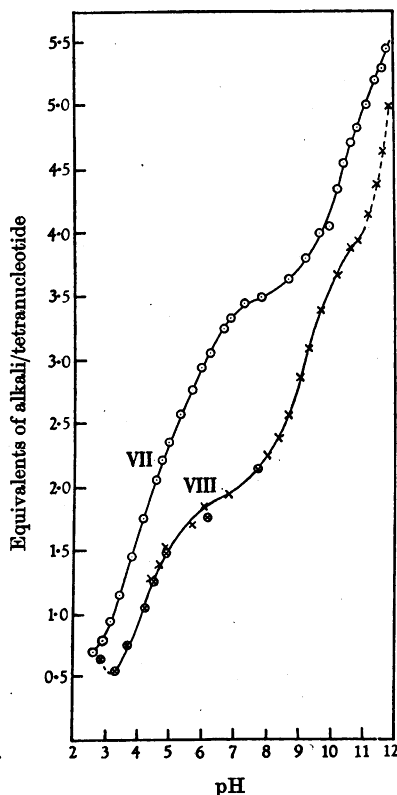
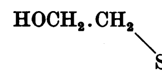
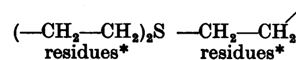


Fig. 3. The electrometric titration of yeast ribonucleic acid (Y5/42), curve VII, ⊙, and its *H* product (HY7/47B), curve VIII, ×⊗.

Table 3. *Numbers of titratable groups/tetranucleotide present in nucleic acids and numbers of groups titratable or blocked in their H compounds*





	Primary phosphoryl	Secondary phosphoryl	Amino	Purine-pyrimidine hydroxyl	Total groups blocked	$(—CH_2—CH_2)_2S$ residues*	$HOCH_2.CH_2$ S $—CH_2—CH_2$ residues*
YNA, Y5/42	2·8	0·8	2·7–3·0	2·0	—	—	—
H-YNA, HY7/47B	1·6	0·4	≮ 1·2	1·8	—	—	—
Groups blocked	1·2	0·4	0–1·8	0·2	1·8–3·6	2·4	2·5
DYNA, DY8	2·5	1·2†	0	3·0	—	—	—
H-DYNA, HDY2/34B	1·2	0·6†	0	3·0	—	—	—
Groups blocked	1·3	0·6†	0	0	1·9	2·0	2·1
TNA, T5	4·0	0	3·0	2·0	—	—	—
H-TNA, HT3/53B	1·4	0·7‡	≮ 1·4	1·0	—	—	—
Groups blocked	2·6	(−0·7)	0–1·6	1·0	3·6–5·2	2·2	2·2
Guanylic acid	1·0	1·0	1·0	1·0	—	—	—
H-guanylic acid HG4/71	0·8	0·55	0·1	0·5	—	—	—
Groups blocked	0·2	0·45	0·9	0·5	2·05	1·85	2·03

YNA = yeast nucleic acid; DYNA = deaminated yeast nucleic acid; TNA = thymonucleic acid.
* Calculated from sulphur analyses; see text. † Includes hydroxyl of xanthine. ‡ See text.

The titration curve of the B product from deoxypentose nucleic acid (T5) of high molecular weight is compared in Fig. 2 with that of a sample of the parent acid which has been treated with mineral acid; this procedure was adopted because the curve for the acid-treated substance differs slightly from that of the original (Gulland, Jordan & Taylor, 1947), and because the B compound was obtained by precipitation with mineral acid. The identity of the 0·7 equivalents of a group titrating between pH 6·0 and 8·4 is obscure; the only likely group titrating in this range is a secondary phosphoryl group, but the dissociation constant of the liberated group ($pK'_a = 7{\cdot}2$) is different from that of the secondary phosphoryl group in thymic acid ($pK'_a = 6{\cdot}4$) (Gulland, Jordan & Taylor, 1947) with which it may suitably be compared.

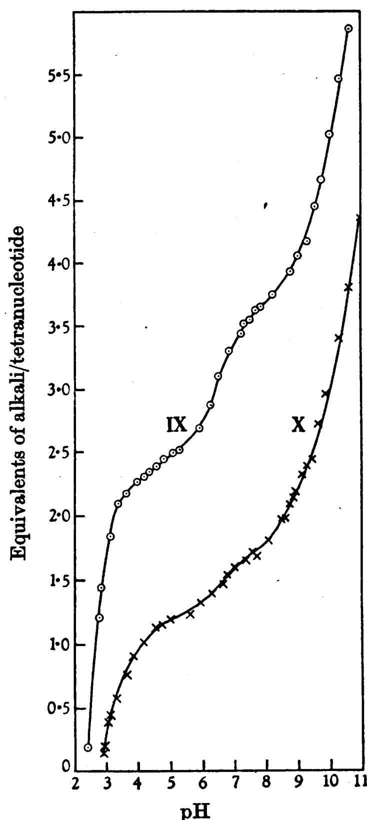


Fig. 4. The electrometric titration of deaminated yeast ribonucleic (DY8), curve IX, ⊙, and its H product (HDY2/34B), curve X, ×.

The dissociation constant of the purine-pyrimidine hydroxyl groups of yeast ribonucleic acid is changed from $pK'_a = 10{\cdot}5$ to 9·4 by interaction of the acid with H. Such changes are not yet fully understood and have been discussed elsewhere (Fletcher, Gulland & Jordan, 1944; Gulland, Jordan & Taylor, 1947).

For the purpose of calculating the number of purine hydroxyl groups in H-guanylic acid, it is assumed that the dissociation constant of these is the same ($pK'_a = 9{\cdot}5$) as that of guanylic acid; owing to the extensive removal of H residues by hydrolysis above pH 10, it was not possible to determine the complete titration curve.

The carbohydrate hydroxyl groups do not titrate below pH 12, and hence it was not possible to determine the extent, if any, of their reaction with H by electrometric titration.

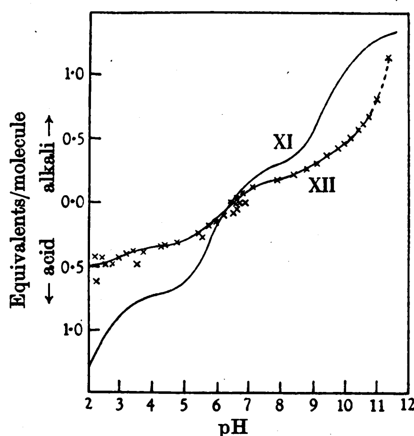


Fig. 5. The electrometric titration of guanylic acid, curve XI (theoretical curve), and its H product (HG4/71), curve XII, ×.

It is not possible to determine by titration alone the extent of the reaction of H with the primary phosphoryl group of guanylic acid. This can be calculated, however, on the assumption that no phosphorus is lost during the reaction. Analysis of the H-guanylic acid shows that the atom ratio P : Na is 1 : 1·2; thus there are in all 1·2 primary and secondary phosphoryl groups neutralized at pH 6·4 which is the pH of the H-guanylic acid solution in water. On titration with alkali (curve XII, Fig. 5) a further 0·15 equivalent of a secondary phosphoryl group is titrated. The total number of secondary and primary phosphoryl groups which have not reacted with H is thus 1·35. Since the titration shows that of these, 0·55 equivalent is due to secondary phosphoryl dissociation, it follows that 0·8 equivalent of a primary phosphoryl group has not reacted with H and hence 0·2 group has reacted. The total number of titratable groups blocked is 2·05.

DISCUSSION

Nucleic acids contain various groups which might be expected under suitable conditions to react with H. Assuming the generally accepted polynucleotide structure of the acids, the number and nature of these groups are summarized in Table 3, which is

based on the polymerization of a statistical tetranucleotide.

Of the various types of reaction into which *H* can enter, that in which the chlorine atoms are replaced by other substituents seems the most probable in the present circumstances, i.e. of the Hughes-Ingold S_N1 type (Watson, 1938; Hughes, 1941). *H* enters into such reactions with amino groups (Clarke, 1912; Helfrich & Reid, 1920; Cashmore & McCombie, 1923; Lawson & Reid, 1925; Ford-Moore, 1940; Boursnell, Francis & Wormall, 1946; Moore, Stein & Fruton, 1946), phenolic anions (Helfrich & Reid, 1920; Ford-Moore, 1940) and carboxylic anions (Doering, Linstead & Ball, 1942; Moore *et al.* 1946), but not with the undissociated carboxyl group (Cohen & Harris, 1942). Inspection of the titration curves of nucleic acids and nucleotides shows that in solution at pH 7 the phosphoryl groups are in the ionic form and the amino groups undissociated, and are thus likely to react with *H*. The purine-pyrimidine hydroxyls $(\text{—N}=\overset{|}{\text{C}}\text{—OH})$ titrate over the range pH 8·0–11·5, being thus similar to phenols in character, and would not be expected to react at pH 7. In DYNA, one hydroxyl group of xanthine is partly dissociated at pH 7 and should therefore react with *H*, whereas the other hydroxyl does not titrate at reactions more acid than pH 12. The sugar hydroxyls have $pK'_a = 13$ and would therefore not be expected to react at pH 7.

The number of titratable groups blocked by *H* is compared with the number of *H* residues calculated from the sulphur analyses (Table 3). It will be seen that in general the results agree with those predicted on theoretical grounds, although the numbers of groups reacting could not always be precisely determined. The only marked divergence is the reaction of the purine-pyrimidine hydroxyl in TNA and guanylic acid; this was unexpected but no satisfactory explanation can be offered.

In the B product from DYNA (HDY 2/34 B) and in the *H* compound from guanylic acid (HG 4/71), it is evident that within experimental error the number of *H* residues is equal to the total number of titratable groups blocked. It is therefore concluded that each titratable group blocked is substituted by one *H* residue. It seems probable, although not certain, that this residue is in the form

$$\text{—CH}_2.\text{CH}_2.\text{S}.\text{CH}_2.\text{CH}_2.\text{OH},$$

since none of the products contained chlorine. It is considered unlikely that *H* has attacked simultaneously a titratable group and a non-titratable hydroxyl, because these latter would not be expected to undergo alkylation at pH 7. Two criticisms of this conclusion should be mentioned, although neither is regarded as likely to be valid. First, it is possible that half of the reacting *H* molecules have cross-linked non-titratable groups and the other half entered into cross linkages with titratable groups; such circumstances, although in agreement with the analytical results, are considered not only to be statistically improbable but also unlikely to occur at pH 7. Secondly, there is the possibility that the reactions with *H* are more complex in character and resemble the formation and decomposition of sulphonium salts known to occur between *H* and its substituted derivatives (Bell, Bennett & Hock, 1927). This reaction mechanism is considered to be most improbable since the formation of sulphonium salts is exceedingly slow at room temperature, and also since no 'sulphonium' dissociation was detected by electrometric titration. It is also possible that *H* has reacted with the amino groups to form 1:4-thiazan derivatives, a type of reaction observed under more vigorous conditions by Clarke (1912), Helfrich & Reid (1920), Lawson & Reid (1925) and Boursnell *et al.* (1946). Our data do not permit a decision as to the nature of the reaction undergone by the amino groups, and it is noteworthy that Moore *et al.* (1946) were unable to establish whether the amino groups of amino-acids and peptides formed secondary amines or thiazans in their reaction with *H*.

The precise figure for the total number of groups blocked in *H*-YNA (HY 7/47 B) could not be ascertained by electrometric titration, but since the number of the primary and secondary phosphoryl and purine-pyrimidine hydroxyl groups blocked is similar to that in *H*-DYNA (HDY 2/34 B), it appears unlikely that any extensive cross linkage has occurred.

In the B product from TNA, on the other hand, it is clear that the number of blocked titratable groups is greater than the number of *H* residues in the molecule, indicating that *H* molecules have reacted with two titratable groups, either in the same molecule or by a process of cross linking between polynucleotide chains. The comparison of viscosities of solutions of TNA before and after treatment with *H* provides additional evidence in favour of some measure of intermolecular cross linkage in view of the increased viscosity of the treated sample.

The calculated analytical and titration data for complete and no cross linking by *H* are compared with the observed data for *H*-guanylic acid in Table 4. The evidence confirms the theory that each titratable group is attached to one *H* residue and that there is little or no cross linking. Such a comparison is not satisfactory in the case of the nucleic acids as the calculated values for complete cross linking and no cross linking are too close together to permit distinction by analysis and titration.

The formation of two series of products, not one, by the interaction of nucleic acids with *H* at pH 7

must now be considered. The salient facts are as follows: (i) both A and B products were free from chlorine and contained unsubstituted phosphoryl groups, the members of the A series being sparingly soluble sodium salts (Table 2), dissolving only at reactions more alkaline than pH 10, whereas the B products were precipitated by mineral acid from solutions of their sodium salts; (ii) the ratio 'yield of A/yield of B' in any given instance increased with longer duration of reaction and higher molecular ratios of *H*/tetranucleotide, but was practically independent of the volume of the reaction mixture (Table 1); (iii) there was no marked or constant difference in the sulphur contents of the A and B products (Table 2); (iv) further treatment of a B compound gave rise to a new pair of A and B products, both containing considerably more sulphur (Table 2, HHDY 3/43 A/B). It is therefore clear that related members of the A and B series were not identical, and that the B products were not direct precursors of the A products formed simultaneously and having approximately the same sulphur content.

Table 4. *Comparison of observed and calculated data for H-guanylic acid* (*HG* 4/71)

Analysis	Calculated for no cross linkage	Calculated for complete cross linkage	Observed
No. of groups blocked/mol.	2·03	1·85	2·05
(%)			
C	36·2	38·1	37·0
H	4·9	4·9	4·7
N	11·7	12·8	11·8
P	5·2	5·7	5·3
S	10·8	10·8	10·8
Na	4·6	5·0	4·7

Two explanations of these differences may be mentioned, and these could operate singly or simultaneously. It is possible that the A products have been produced by the cross linking by *H* of lyophilic groups on the same polynucleotide chain; thus two solubility-conferring groups would be rendered inactive for each atom of sulphur introduced. Alternatively, the A products may have a much higher molecular weight than the B products as a result of the cross linking occurring between different polynucleotide chains. The B products are considered to be mainly the monosubstituted derivatives of thiodiglycol (see above). These hypotheses are in conformity with the possibility that B compounds on further treatment with *H* can produce either B compounds of higher sulphur content or by cross linking, A compounds of higher sulphur content (see (ii) and (iv) above).

There is thus ample evidence that at the pH values pertaining in tissues both deoxypentose nucleic acid of calf thymus and yeast ribonucleic acid will react with *H*. It is to be emphasized that it is not known whether the structure of pentose and deoxypentose nucleic acids of all tissues are the same as or different from those of YNA and TNA (Gulland, 1944, 1947; Gulland *et al.* 1945; Davidson & Waymouth, 1944–5), but there is no evidence to suggest that the reactive groups present in nucleic acids from other sources are not similar to or identical with those of YNA and TNA. The biological implications of the results are clear. It is generally agreed that deoxypentose and pentose nucleic acids are constituents of all nuclei, and are involved in the mechanism of mitosis. The attack of cell nucleic acids by *H* would interfere with this mechanism and hence with the proliferation of tissues injured by *H*. The present investigation thus provides a chemical parallel to and possibly an explanation of the cytological observations of Koller (1942), Robson (1943) and Fell & Allsopp (1943).

SUMMARY

1. It has been found possible to induce reaction between nucleic acids, guanylic acid and mustard gas (*H*) under mild conditions of pH and temperature.

2. The products of the reaction between nucleic acids and mustard gas have been isolated and analyzed. They are found to contain sulphur but not chlorine.

3. Electrometric titration of the *H* compounds shows that the primary and secondary phosphoryl groups, and the amino groups have in every case, when present, reacted with *H*. The purine-pyrimidine hydroxyl groups have been found to react only in certain cases.

4. The viscosity of solutions of the *H* compound of a high molecular weight sample of deoxypentose nucleic acid was found to be much greater than that of the original acid.

5. Possible structures of the *H* compounds are discussed, and the significance of the results considered in relation to the delayed healing of mustard gas lesions.

It is a pleasure to record our thanks to the Chief Scientist, Ministry of Supply, for permission to publish these results; to Mr J. E. Still for performing a number of the microanalyses; to Mr J. M. Creeth for the determinations of viscosities; to Mr W. E. Fletcher for the titration curve of DY 8; to Imperial Chemical Industries Ltd. for the loan of apparatus; and to the British Empire Cancer Campaign for a maintenance grant to one of us (D. T. E.) during a short period at the end of the work here recorded.

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The Component Acids of Herring Visceral Fat

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The component acids of North Sea herring flesh fats laid down at different seasons, including those of intensive feeding when much fat is deposited, and those of relative inanition when the body fat content falls to a low level, have been discussed by Lovern (1938). Similar data for the flesh fat of Icelandic herring have been given by Bjarnason & Meara (1944). The component acids of fats from other parts of the herring have not been studied in detail hitherto. Some time ago Mr J. R. Bruce, of the Marine Biological Station, Port Erin, Isle of Man, sent to us a quantity of oil extracted from the viscera of herrings taken locally, and we have now had an opportunity to examine the nature of the component acids of the visceral fat, which displays interesting differences from the characteristic composition of herring flesh fats.