EFFECT OF IONIZING RADIATION ON AQUEOUS SOLUTIONS OF GUANYLIC ACID AND GUANOSINE

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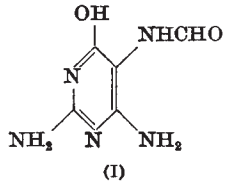
WHEN an aqueous solution of guanylic acid (4×10^{-3} *M*), irradiated with high-energy electrons (approximately 4×10^6 r.), is analysed by paper chromatography, using an acid solvent, two ultra-violet absorbing products are found. One product, obtained in lower yield, has R_F values identical with guanine in three solvent systems. Its ultra-violet spectra at *p*H 7 and 11 are also identical with those of guanine. The second product (*GA*-α) has ultra-violet spectra as shown in Fig. 1, and R_F values as shown in Table 1. The spectra were similar to those for products of the type *M*-2, obtained by the alkylation of guanylic acid under mildly alkaline conditions as described by Lawley and Wallick[1]. Dr. P. D. Lawley suggested that both alkylating agents and radiation produce fission of the imidazole ring, yielding, in the case of radiation, 2 : 4-diamino-5-formamido-6-hydroxypyrimidine (I). This compound was prepared by Mr. G. M. Timmis and its ultra-violet spectra determined by Dr. Lawley. The spectra of I (Fig. 1) and its R_F values in three solvent systems (Table 1) were found to be identical with those for *GA*-α.



(I)

If a solution of guanylic acid is buffered at *p*H 7 (0·02 *M* phosphate buffer) during irradiation and analysed by paper chromatography, using a neutral solvent[3], the second product appears at R_F 0·73. This product (*GA*-β) has ultra-violet spectra as shown in Fig. 2. *GA*-β hydrolyses rapidly in acid. In 0·1 *N* hydrochloric acid, at 37° C., the ratio $D_{260m\mu}/D_{275m\mu}$ increases with a half-life of 4 hr. The hydrolysate had a single optical density maximum at $\lambda = 265$ mμ. No spectral change was observed for at least 19 hr. when guanylic acid was incubated under identical conditions of temperature and *p*H. The orcinol test, for sugar, gave a positive colour with *GA*-β but not with *GA*-α. This suggests that *GA*-β is *GA*-α with a sugar moiety, possibly ribose-phosphate, attached. This shows that attack on the imidazole ring is the primary effect of irradiation.

Similar results have been obtained on irradiation of guanosine in aqueous solution. Analysis by paper chromatography gives two ultra-violet absorbing products. One product has been identified with guanine. Using an acid solvent, the second product (*GS*-α) has R_F values identical with those of I in two solvent systems[2,4]. Its ultra-violet spectra at *p*H 1 and 7 are identical with those of I. If a neutral solvent is used, the second product (*GS*-β) has an R_F value of 0·70 in ammonium sulphate/*iso*propanol[3]. *GS*-β has ultra-violet spectra at *p*H 7 and 11·5 similar to those for *GA*-β. In acid, *GS*-β hydrolyses rapidly, the ratio $D_{260m\mu}/D_{275m\mu}$ increasing with a half-life of 8 hr. in 0·8 *N* hydrochloric acid at room temperature. On hydrolysis the ultra-violet absorption maximum shifted to $\lambda = 263$ mμ. Guanosine incubated under the same conditions showed no change of spectrum for at least 19 hr. The orcinol test gave a colour with *GS*-β, but not with *GS*-α. This suggests that *GS*-β is *GS*-α with ribose attached.

Table 1

Solvent system	Ref.	R_F (*GA*-1 and I)
*Iso*butyric acid–ammonia	2	0·48
Ammonium sulphate–*iso*propanol	3	0·43
Methanol–hydrochloric acid	4	0·21

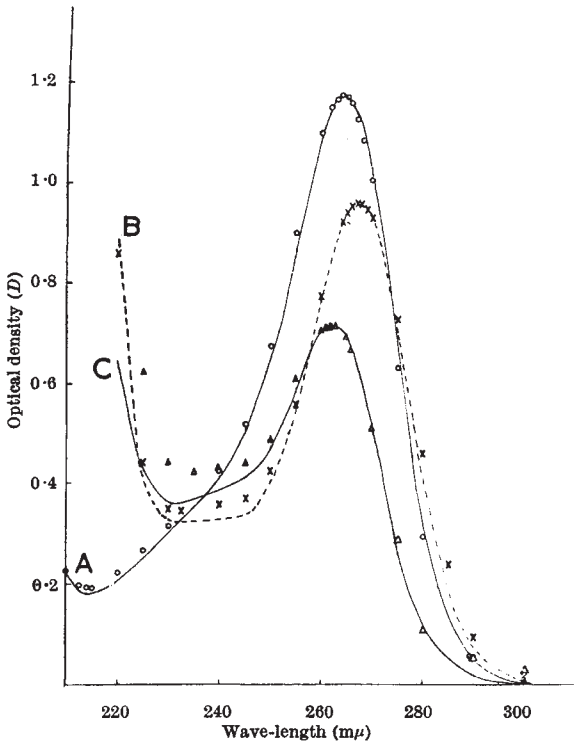


Fig. 1. Ultra-violet absorption spectra for I and *GA*-α. Plotted points apply to *GA*-α, and curves apply to I, adjusted to the ultra-violet absorption maxima for *GA*-α. Curves *A*, *B*, *C* (points ○, ×, △) refer to *p*H values 0·1, 7 and 12, respectively.

The radiation degradation of guanylic acid and guanosine is strikingly different from that of the corresponding adenine derivatives, where adenine is the sole ultra-violet absorbing product[5]. These results suggest that the guanine/adenine ratio of

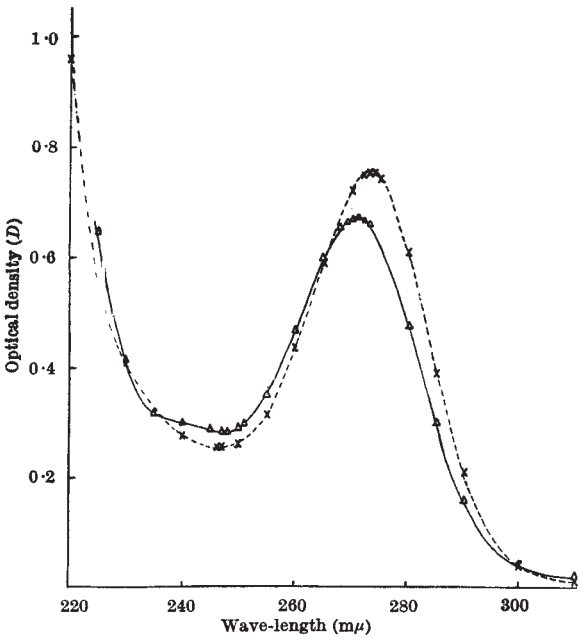


Fig. 2. Ultra-violet absorption spectra for *GA-β* determined at *p*H 7 (× - - - ×) and *p*H 11·5 (△——△)

nucleic acids may determine to a considerable extent the effect of ionizing radiation upon them.

Observations on the effects of alkylating agents have shown the imidazole ring of deoxyguanylic acid to be the most reactive portion of the constituent nucleotides of deoxyribonucleic acid[6], and that this alkylation labilizes the deoxyriboside linkage[7]. These results, therefore, show a close analogy with the effects of ionizing radiation in that, in both cases, the imidazole ring is attacked with consequent labilization of the glycosidic linkage with the purine. Since these comparisons could also apply to *in vivo* conditions, they could be held to provide a plausible explanation of at least some part of the radiomimetic properties of biological alkylating agents.

Radiation effects upon model compounds are being investigated in conjunction with Mr. G. M. Timmis and Dr. C. Leese. A quantitative comparison of the reactivities of purine and pyrimidine nucleotides to ionizing radiation will be made. It has already been reported that the change, with oxygen tension, of the decrease in optical density produced by ionizing radiation is positive for adenine, but negative for guanine[5].

I wish to thank Prof. J. Rotblat, Physics Department, St. Bartholomew's Hospital, for his kind permission to use the linear accelerator, also Dr. H. C Sutton for his assistance in performing the irradiations. The interest of Prof. J. E. Roberts is much appreciated.

[1] Lawley, P. D., and Wallick, C. A., *Chem. and Indust.*, 633 (1957).
[2] Pabst Laboratories Circular *OR*-7 (1955).
[3] Markham, R., and Smith, J. D., *Biochem. J.*, **49**, 401 (1951).
[4] Kirby, K. S., *Biochim. Biophys. Acta*, **18**, 575 (1955).
[5] Barron, E. S. G., *Rad. Res.*, **1**, 410 (1954).
[6] Lawley, P. D., *Biochim. Biophys. Acta*, **26**, 450 (1957).
[7] Lawley, P. D., *Proc. Chem. Soc.*, 290 (1957).
[8] Hems, G., and Eidinoff, M. L., *Radiation Res.* (in the press).

DISTENSION OF THE RUMEN AND SALIVARY SECRETION

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TWO hypotheses regarding the possible part played by saliva in the condition known as 'bloat' have been made. Weiss[1] suggested that when lush green leguminous forages are fed, lack of fibre led to lack of salivary secretion and on account of this the rumen contents were viscous so that gas easily became trapped as a foam and could not be excreted by belching. The gist of Weiss's hypothesis is that a large salivary secretion helps to prevent bloat. Johns[2], on the other hand, found that cows fed on red clover hay were still liable to bloat, and pointed out that the low surface tension of bovine saliva found by Reid and Huffman[3] made saliva into a foam-promoting agent which could be expected to have the opposite effect to that postulated by Weiss. In support of Johns's hypothesis Mangan[4] has found that when mixed bovine saliva is aerated a very stable foam is formed.

There is little information to show whether or not the salivary secretions of the ruminant are affected by distension of the rumen, and the experiments summarized here were undertaken to investigate this matter. Previously, Coates, Denton, Goding and Wright[5] had reported that inflation or deflation of the rumen of merino sheep had no effect on the rate of secretion of the parotid glands.

Adult sheep, or calves 5–8 months old, were anæsthetized with chloralose (50 mgm./kgm. body-weight). The trachea and rumen were cannulated and the cervical œsophagus ligated. Parotid saliva was collected by cannulating the parotid duct close to its papillæ to avoid damage to the nerve supply to the gland; in later experiments with calves both parotid ducts and both submaxillary ducts were cannulated, while the residual saliva flowing from the mouth and nose was collected separately. The parotid flow in sheep was recorded by a drop counter on a kymograph. Later, in calves, the volumes of saliva secreted from each pair of glands were measured in two calibrated siphons, the hydrostatic pressure changes in which were recorded by pressure transducers and a Sanborn recorder (Reid, in preparation). The rumen was inflated to known pressures by carbon dioxide, nitrogen and occasionally oxygen or air.