Also heat-denatured DNA irradiated in presence of daunomycin and worked out in the same experimental conditions described for native DNA, formed a stable combination with daunomycin (Table II).

The observations reported in this communication are only qualitative; and at this stage in the investigation no attempt was made to characterize the complex that is formed by photoirradiation and no conclusion can be drawn with respect to the mechanism of the reaction. Studies are in progress to elucidate whether daunomycin reacts with free purine and pyrimidine nucleosides or nucleotides. Preliminary experiments with apurinic and apyrimidinic DNA (Figure 3) show that only with apyrimidinic DNA it is possible to obtain after photoirradiation a stable combination with daunomycin showing a spectrum similar (curve 1) to that of daunomycin-DNA irradiated complex. This suggests that purinic bases may be reactive sites of DNA.

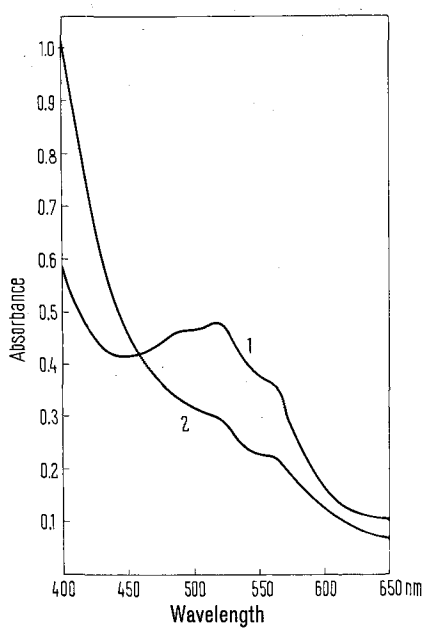

Fig. 3. Spectra of apyrimidinic DNA-Daunomycin 1) and apurinic DNA-Daunomycin irradiated complexes 2). Apurinic and apyrimidinic DNA's were obtained according to TAMM[8] and SHAPIRO[9] respectively. In this experiment a PCQ-XI photochemical lamp (Ultraviolet Products) was used. A solution of DNA (about $8 \times 10^{-3} M$ of nucleotide phosphorus) and of Daunomycin (2×10^{-4}) in $0.01 M$ Na phosphate (pH 6.8) was irradiated for 15 min. The spectrum was recorded after phenol extraction.

Daunomycin-DNA complexes are irradiated in the UV region so that both bases and daunomycin are excited. The obvious possibility of direct damage to DNA has as yet to be established. Using visible light so that only daunomycin is excited, preliminary experiments indicated that, besides direct damage to DNA, a stable combination of daunomycin with native DNA took place. The possibility may exist that, in the case of photoinactivation of viruses[5,6], reaction mechanisms similar to photochemical binding may be involved. In conclusion, the unusual strength of the photochemical binding of daunomycin to DNA raises the possibility of the formation of a covalent bond between daunomycin and nucleic acids, as a consequence of irradiation. The results observed could have interesting implications for the explanation of the biological effects of daunomycin.

Riassunto. Quale effetto dell'irradiazione UV è stato osservato un legame insolitamente forte tra daunomicina ed acidi nucleici, che suggerisce la possibilità della formazione di un legame covalente.

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Morphogenic Effects of Halogenated Thymidine Analogs on *Drosophila* III. 5-Iododeoxyuridine[1]

When *Drosophila* larvae are fed a mixture of 5-bromodeoxyuridine (BUdR) and 5-fluorouracil (FU), the hatching adult flies show a variety of developmental lesions including instances of supernumerary tissue growth as well as bristles in place of hairs[2,3]. Under similar conditions of treatment, FU is ineffective in stimulating growth while BUdR administered alone induces a low frequency of developmental modifications. These observations suggest that treatment with BUdR is the prime factor in upsetting normal growth processes in *Drosophila* while FU amplifies this effect.

BUdR, a thymidine analog, is incorporated into DNA, and the level of its incorporation can be increased by inhibiting de novo synthesis of thymidine monophosphate. 5-Fluorodeoxyuridine is a potent inhibitor of thymidylate synthetase[4], and has been used to create thymidine deficient conditions in a number of biological systems[5-7]. Since the presence of FU increased the amount of BUdR incorporated into *Drosophila* DNA[8], presumably one of the roles of FU following ingestion by *Drosophila* larvae is the inhibition of thymidylate synthetase. In order to

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evaluate the effects of thymidine substitution on the growth and differentiation of *Drosophila*, a study paralleling that with BUdR was undertaken using the thymidine analog 5-iododeoxyuridine (IUdR) in the presence and in the absence of FU. This report indicates that with respect to the parameters tested, both the morphological effects and the incorporation of IUdR into *Drosophila* DNA are augmented by the presence of FU.

The methods of analog feeding and classification of growth modifications were the same as those used in previous studies with BUdR[3] except the treatment interval was extended to 8 h. The lesions obtained with IUdR + FU resemble those induced by BUdR + FU, and include supernumerary growths as well as bristle modifications. Examples of these developmental modifications are presented in Figure 1. The category designated as single bristle effect is not depicted since it is represented by the appearance of an isolated bristle variant similar to those illustrated in cluster formation.

Frequency data on morphological response has been limited to examination of the wings of treated specimens, and Table I summarizes this data. Administration of IUdR induces a few bristle events, and this frequency is markedly increased by the use of FU with IUdR. Additional indication of extensive developmental modification is the appearance of clusters and supernumeraries in the IUdR + FU treated specimens. Since more than one lesion may be induced in a single wing, comparison of lesion frequency in these experiments has been expressed on the basis of total lesions induced per total wing sample. Approximately the same level of response was obtained within the concentration range of 0.115 to 0.460 mg/ml IUdR.

The procedure detailed by RITOSSA et al.[9] was followed for isolating DNA from *Drosophila* larvae fed ^{3}H-IUdR and ^{3}H-IUdR + FU. For each of the experiments, larvae from a single collection period were washed and divided into 2 groups, one receiving the radioisotope with FU and the other receiving only ^{3}H-IUdR. The feeding interval for the first series was 12 h while the second experiment was concluded after 8 h of analog ingestion. [6-^{3}H]-IUdR (Schwarz/Mann) was added to an aqueous solution of 0.2 mg/ml IUdR to give a final concentration of 11.5 μC/0.59 μmol/ml. The concentration of the DNA samples was estimated by the diphenylamine reaction (BURTON[10]) using a sample of calf thymus DNA as a standard, and radioactivity was determined on triplicate aliquots of each sample which were counted twice to obtain an average of the counts per minute. Aliquots were also precipitated following DNase digestion to confirm incorporation of the ^{3}H-IUdR into DNA; a 93% loss of radioactivity was obtained following DNase treatment of each of the samples. In both experiments, increased incorporation of IUdR was obtained when FU was present during the feeding interval (Table II).

The 4 DNA samples were then centrifuged to equilibrium in CsCl following the procedures detailed previously[8]. Optical density profiles together with the corresponding radioactivity of each fraction are presented in Figure 2. The buoyant density of the DNA was estimated by determining the density of the CsCl of selected fractions from one gradient of each DNA sample using refractometric measurements; this information has been included for gradient B_2. A single peak of UV absorbing material appears in all gradients, and the buoyant density of this material as estimated by refractometric measurements agrees with that presented previously for normal *Drosophila* DNA[8]. In the IUdR samples, radioactivity parallels the main optical density peak with some indication of IUdR-DNA on the dense side of the optical density peak. In the IUdr + FU samples, there is a pronounced shift in the radioactivity toward the denser regions of the gradient but some IUdR-DNA remains distributed to the less dense side of the optical density peak.

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Table I. Morphogenic response to 5-iododeoxyuridine

Analog concentration (mg/ml)		Wings affected/total	Type of lesion				Lesion frequency (Total lesions/Total wings)
IUdR	FU		Single	Cluster	Supernumerary	Total	
0.115	–	2/144	2	0	0	2	0.014
0.115	0.150	84/250	45	20	56	121	0.484
0.230	–	3/289	3	0	0	3	0.014
0.230	0.150	92/293	43	24	66	133	0.454
0.460	–	7/393	7	0	0	7	0.018
0.460	0.150	69/245	26	17	54	97	0.396

Table II. Incorporation of ^{3}H-IUDR into *Drosophila* DNA

Treatment	DNA (μg)	Counts per min	CPM/μgDNA	Ratio (B/A)
1. A) IUDR	13.2	463	35.1	1.57
B) IUDR + FU	19.0	1045	55.0	
2. A) IUDR	26.2	1147	43.8	1.13
B) IUDR + FU	21.4	1060	49.5	

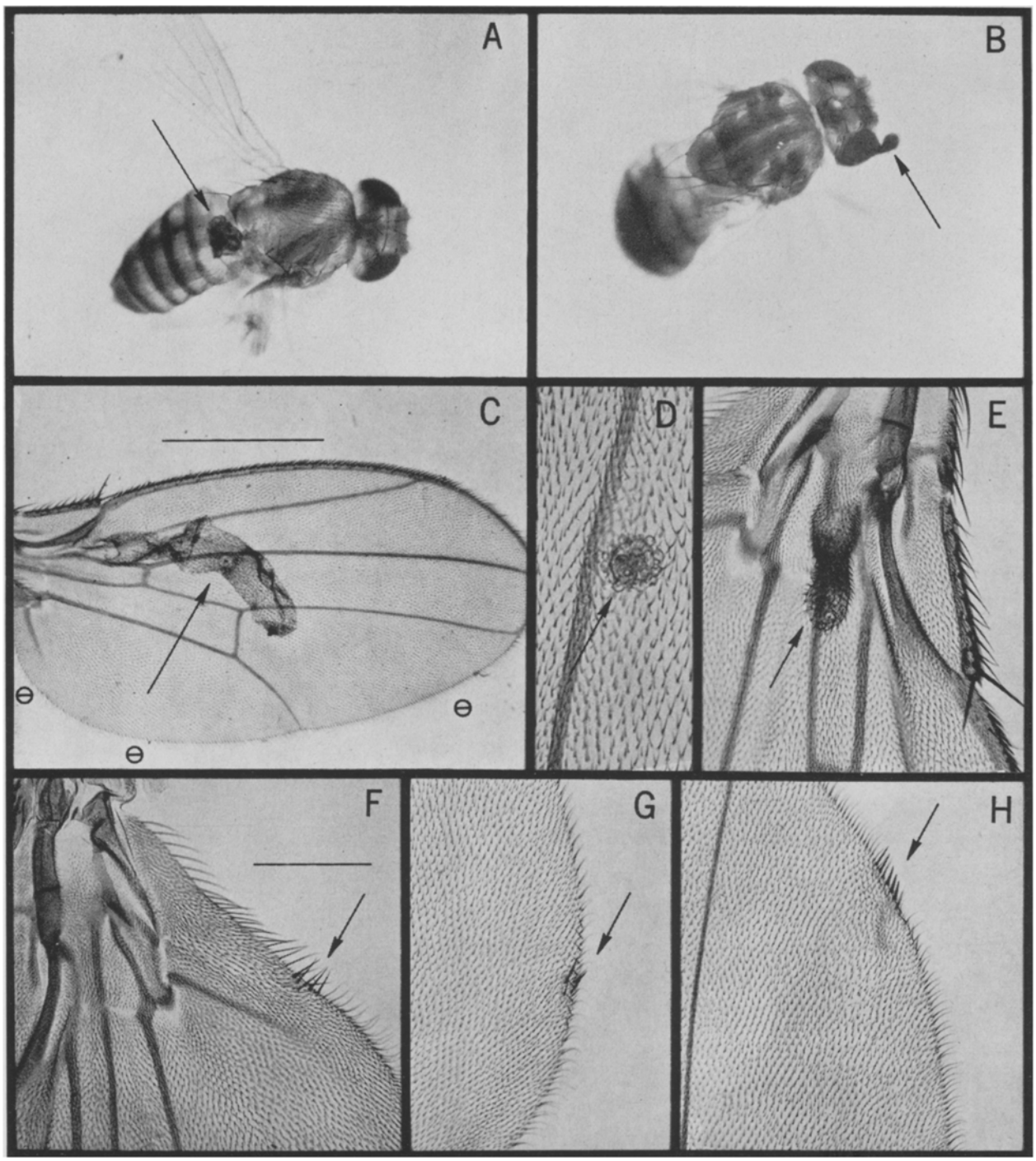

Fig. 1. Lesions induced by IUDR + FU treatment. A) A small supernumerary wing is indicated in the dorsal thoracic region. B) A club-shaped supernumerary structure from the eye. Eye pigment and facet development is continous with the rest of the eye. C) Supernumerary wing development on a wing. Note that the other structures of the wing including bristle patterns and wing veins are normal except for the extra growth indicated by the arrow. D) A small supernumerary structure of the type which is most often encountered by the analog treatment. E) A supernumerary structure showing extensive bristle development. F) G) H) Three different wings with examples of clusters among the fine wing hairs. The position of these bristle clusters would correspond to the points indicated in Figure C) proceeding from left to right respectively. (Magnification represented by the bar in Figure C) is 0.5 mm and in Figure F) 0.2 mm. Figures E), F), G) and H) are the same magnification while Figure D) is enlarged 2.5 X Figure E).

All 3 categories of growth modifications were obtained by high doses of BUdR while low doses induced primarily single bristle alterations[3]. In the present study, concentrations of IUdR equimolar to the lower ranges tested for BUdR induced single bristle events. The frequency of lesion induction as well as the degree of developmental modification is considerably less than when the thymidine analogs are administered with FU, but the conditions of administration of the thymidine analogs either with or without FU are not associated with lethality of the treated

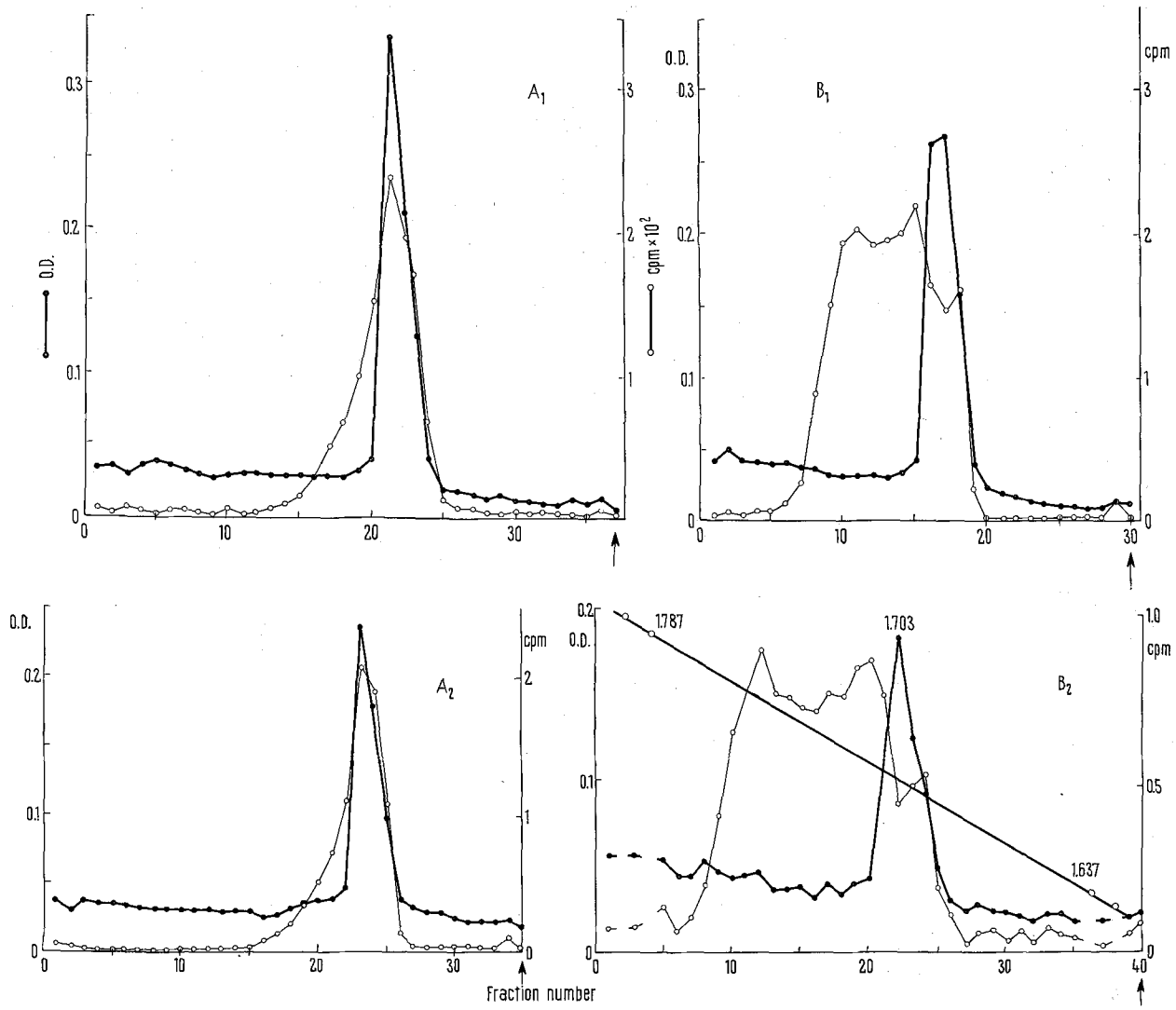

Fig. 2. Cesium chloride density gradient centrifugation of *Drosophila* DNA obtained from larvae exposed to ^{3}H-IUdR (A) and ^{3}H-IUdR + FU (B). A_1 and B_1 were isolated after an 8 h feeding interval on the analogs, and DNA in set A_2 and B_2 was isolated after 12 h.

individuals. The incorporation of the thymidine analogs IUdR and BUdR as well as exogenous thymidine[8] into *Drosophila* DNA are increased in the presence of FU; this difference in utilization of exogenous nucleosides thus appears to be consistent for these conditions of administration. On the other hand, the increase in the incorporation of both IUdR and BUdR into *Drosophila* DNA achieved with the use of FU is small and it seems unlikely that a slight concentration difference in IUdR or BUdR incorporation could account for the pronounced differences in developmental effects between larvae treated in the presence and in the absence of FU. Maximum developmental response was obtained when BUdR and FU were offered simultaneously to *Drosophila* larvae; treatment with the two analogs in sequence is not highly effective[3]. If the modified growth patterns in *Drosophila* are related to the incorporation of IUdR and BUdR into DNA, then differences in the sites of analog incorporation into DNA in the presence and in the absence of FU might account for the difference in morphogenic response obtained under the two conditions of administration. SIMON[7] and TOLIVER et al.[11] have proposed that some thymine sites in the DNA of HeLa cells will accept BUdR more readily than others, and that the acceptance of BUdR into less preferred sites might be favored by raising the level of BUdR in the cell through the use of FUdR. The CsCl gradients of IUdR and IUdR + FU samples of *Drosophila* DNA indicate differences in incorporation of the thymidine analog under the two conditions of administration.

Zusammenfassung. Die Häufigkeit der durch 5-Iododeoxyuridin (IUdR) bei *Drosophila* induzierten Abnormalitäten kann durch gleichzeitige Fütterung der Larven mit 5-Fluorouracil (FU) erhöht werden. Die Menge des in die *Drosophila*-DNS inkorporierten IUdR ist bei Anwesenheit von FU höher; die Verteilung dieser IUdR-DNS im CsCl-Dichtegradienten ist verschieden von der in Abwesenheit von FU synthetisierten DNS.

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