The effect of spermine on the spontaneous reversion of a base-analogue induced (*N*11) and an acridine induced (*FC*47) *r*11 mutant of the *Escherichia coliphage T*4

*N*11	Lysates prepared from an initial average phage titre of 3.6/ml									
Spermine concentration	–					250 µg/ml				
Lysates	*C*1	*C*2	*C*3	*C*4	Average of *C*1, *C*2, *C*3, *C*4	*S*1	*S*2	*S*3	*S*4	Average of *S*1, *S*2, *S*3, *S*4
Terminal phage titre $\times 10^{10}$/ml	1.26	1.45	10.0	14.9	6.9	1.48	4.8	14.4	5.35	6.5
Reversion index $\times 10^{-6}$	0.08	0.09	0.02	0.13	0.08	0.16	0.07	0.16	0.19	0.14

*FC*47	Lysates prepared from an initial average phage titre of 2.5/ml									
Spermine concentration			–			250 µg/ml				
Lysates	*C*1	*C*2	*C*3	*C*4	Average of *C*1, *C*2, *C*3, *C*4	*S*1	*S*2	*S*3	*S*4	Average of *S*1, *S*2, *S*3, *S*4
Terminal phage titre $\times 10^{11}$/ml	1.85	3.6	2.5	3.2	2.77	5.6	4.4	2.2	1.74	3.48
Reversion index $\times 10^{-6}$ for normal wild type plaques	0.55	0.66	0.52	0.53	0.56	0.26	0.40	0.62	0.53	0.54
Reversion index $\times 10^{-6}$ for tiny plaques	0.51	0.21	0.15	0.27	0.29	0.13	0.13	0.22	0.38	0.22
Overall reversion index $\times 10^{-6}$	1.06	0.87	0.67	0.80	0.85	0.39	0.53	0.84	0.91	0.67

E. coli BB was grown in glucose-salts (*M*9) medium to ca. 2×10^8 cells/ml and infected with a low multiplicity of the mutant 'phage to reduce the chance of introducing revertants already present in the mutant stock. Lysis was allowed to proceed overnight; since the reversion index in such experiments is subject to large fluctuations due to clonal growth of revertants, 4 separate lysates were prepared for each estimation. 'Phage titres in the lysates were determined on *E. coli B*; *E. coli K* (lysogenic for λ prophage) was used as the selective strain for the detection of revertants. The frequency of revertants is expressed as the reversion index[8], that is, the proportion of wildtype particles present in the lysate.

The Table illustrates the results obtained for these mutants in the absence and presence (250 µg/ml) of spermine. No significant effect of spermine is discernible on the terminal titre of the lysates or on the spontaneous reversion of either mutant. The 2 mutants chosen for study are representative of the 2 major classes of simple (revertible, by definition) mutational changes which occur in 'phage DNA[9], the base-analogue type transitional change and the acridine type frame-shift change, and spermine would appear to offer no protection against spontaneous reversion of such changes in 'phage[10].

Résumé. La spermine n'offre aucune protection contre la réversion des mutants de *r*II chez *Escherichia coliphage T*4, quoiqu'on ait constaté qu'elle antimutagénique chez *E. coli*.

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[9] D. R. KRIEG, *Progress in Nucleic Acid Research* (Ed. J. DAVIDSON and W. E. COHN; Academic Press Inc., New York 1963), vol. II, p. 125.
[10] I thank Professor J. M. Thoday for supplying the laboratory facilities, and the British Empire Cancer Campaign for Research for financial support.

Thymidine Teratogenesis and Mutagenesis in *Drosophila melanogaster*

KAPLAN and SISKEN[1] and STRÖMNAES[2] reported on the mutagenic effect of ^{3}HT (tritiated thymidine) mixed in the larval medium and fed to *Drosophila* larvae, either for a part or full period of the larval life. KAPLAN et al.[3,4] reported that the recessive lethals induced by ^{3}HT show a non-random distribution. The non-random distribution was considered to reflect the varying frequency with which thymidine base occurs along the length of the *Drosophila X*-chromosome.

To what extent the recessive lethals scored are due to the radiation damage caused by the β particles emitted from ^{3}H, is not clear. PERSON and LEWIS[5] suggest that, in strain 15 *Escherichia coli* at least, the mutagenic action

[1] W. D. KAPLAN and J. E. SISKEN, Experientia *16*, 67 (1960).
[2] O. STRÖMNAES, Genetics *4*, 440 (1962).
[3] W. D. KAPLAN, V. E. THINDERHOLT, H. D. GUGLER and K. K. KIDD, Drosoph. Inf. Serv. *37*, 92 (1963).
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stems from effects other than radiation damage. A transmutation effect might also be responsible for the mutation process (resulting in a non-random distribution of lethals), and there are no experiments differentiating the 2 effects. Another possibility is that the induction of sex-linked recessive lethals might to some extent be due to the thymidine itself. It should be mentioned that, with 0.01% of thymidine employed in control experiments, KAPLAN et al.[1] detected no mutagenic effect. With these facts in mind, the present investigation was planned to study the effect of normal thymidine fed to *Drosophila* larvae during the full period of egg and larval development.

Material and methods. The Oregon-R strain, used in this work, has been maintained in our laboratory for years. The medium was prepared according to the formula: sugar 7 g, bran 7 g, maize meal 6 g, and Agar 0.8 g cooked in 100 ml water and 0.4 g of Nipagin dissolved in alcohol, added to it when still cooking and well stirred. About 20–25 g of this medium was transferred into 250 ml culture bottles each. For the preparation of the test medium, the bottles were kept in a hot-water bath and, when the medium became soft, were taken out and the specified amount of thymidine added (temperature about 80–85 °C) and well stirred. The bottles were cooled in a cold water bath. For experiments I and II, 250 and 500 mg of thymidine respectively were added to the specified amount of the medium. For experiment III, 500 mg of thymidine were irradiated with 100,000 r and then added to the medium. The method of irradiation was the same as reported by OM PARKASH[6]. In all the experiments, grade-A thymidine obtained from Calbiochem (Switzerland) was employed. Immediately after cooling, the cultures were started with about 20 pairs of Oregon-R flies/bottle. The parents were discarded after 10–12 days. The cultures were kept at 23 °C.

In order to determine the rate of induced sex-linked recessive lethals, the M-5 (BASC) technique was employed. Upon eclusion, 0–2-day-old males that had developed as larvae on the medium containing thymidine, were individually mated to 3 M-5 females (Edinburgh dual purpose stock) each, and after 10–13 days the bottles were emptied. In all the experiments 10–15 *X*-chromosomes/male were tested. Further, before starting with lethal tests, all the emerging individuals (developed on the test medium), males and females, were checked for any visible morphological abnormalities. For the second part of the experiment, additional cultures were started with the test medium employing the following strains (all at 23 °C) Oregon-R, *w, yw, y/f, ext, y; cn cn; ss* and *bw st/bw st*, 15–20 pairs from each strain were introduced into each bottle separately, left in for about 12 days and then discarded. The progeny *F*1 was checked only for visible morphological abnormalities. This part of the experiment with Oregon-R strain was repeated about 10 times in order to be sure of the effects observed. The larvae were not checked at all. Along with the test cultures, control cultures were started on the normal medium.

Thymidine-induced sex-linked recessive lethals

	No. of *X*-chromosomes tested	Lethals scored	%-rate	Remarks
Control No thymidine	2050	5	0.24	all single
I. 250 mg thymidine (1%)	1072	7	0.66	all single
II. 500 mg thymidine (2%)	1827	18	1.0	9 singles 3 clusters of 2 1 cluster of 3
III. 500 mg thymidine irradiated with 100,000 r	1859	9	0.48	6 singles 1 cluster of 3

Results. Teratogenic effect of thymidine. 1–2% of thymidine added to the normal medium was toxic to the developing larvae, as was evident from a very large number of dead pupae present in the test bottles. Under the present experimental conditions, the following visible abnormalities were observed. (In all the checks with various strains tested, the same abnormalities appeared, either singly or in conjunction with one or more of the other abnormalities, and in various repetetions 30–75% of the individuals, irrespective of sex, were affected. No abnormalities, other than those mentioned below, were ever detected. No checks were made for the abnormalities of the inner organs).

I. Increase in the number of the scutellar bristles, 5–7 instead of 4 occurring normally.

II. Wing-margins clipped, the number, size and position of the cuts varying.

III. Venous anomalies, including interruption of the longitudinal vein (V), interruption of or missing posterior crossvein, presence of an extra (anterior) crossvein between the third and fourth longitudinal veins, plexus, delta and fused-like abnormalities etc.

IV. Irregular abdominal bands, malformation of the abdomen of varying degrees of severity.

V. Deformation of the legs, the degree of deformation and the number of legs affected varying.

In parallel controls, the only abnormalities observed were of type (I), the number of affected individuals being of the order of 1%. Flies with irregularities of the abdominal bands were still more rare and abnormalities of the wings or veins were never observed.

Induction of sex-linked recessive lethals. As mentioned earlier, the lethals were scored by the M-5 technique and checked for stability for a number of generations. The results are shown in the Table.

Discussion. No teratogenic effect of thymidine in *Drosophila melanogaster* or in other organisms has been reported so far. FRITZ-NIGGLI and KLAAS[7] have shown that thymidine can be taken up by various tissue cells of *Drosophila* larvae. However, no morphological abnormalities were reported. The teratogenic effect observed might be due either to mutation of the somatic cells or to some sort of metabolic disturbance caused by thymidine. The exact cause is not clear. Perhaps normal thymidine acts as a base-analogue in the case of *D. melanogaster*. Incidentally, the morphological abnormalities concerning the wings are very similar to those detected by GERSHENSON et al.[8] amongst the flies grown on the medium containing 8–12% of the sodium salt of calf-thymus nucleic acid, though in their case, the percentage of the affected flies was much smaller.

As is apparent from the Table, thymidine at 1% concentration is weakly mutagenic ($Chi^2 = 3.05$, *P*-value lying between 0.05 and 0.1) and at 2% concentration the results are statistically significant ($Chi^2 = 8.89$, *P*-value

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[7] H. FRITZ-NIGGLI and S. KLAAS, Drosoph. Inf. Serv. *34*, 78 (1960).
[8] S. M. GERSHENSON, R. A. SILVERMAN, O. L. LEVOCHKINA, P. O. SITKO and N. D. TARNAVSKIE, Zh. Obshch. Biol. *9*, 69 (1948).

being less than 0.01). The difference for 2 concentrations is not significant. Further, it is evident that irradiation of thymidine diminishes its mutagenic effectiveness. It may be seen from the Table that quite often the lethals appear in bunches. The phenomenon of 'clusters' would indirectly indicate that thymidine is entering the germ cells of the male gonads in the early stages of the larval development. Again the mechanism of the mutagenic action of thymidine is not clear and may be sought in terms of the above considerations; however, this is just a tentative hypothesis[9].

Zusammenfassung. Wird Thymidin dem Standardnährboden von *Drosophila* zugefügt (1–2%), erweist es sich als teratogen. Bei Versuchen mit verschiedenen Stämmen von *D. melanogaster* traten in allen Fällen nach Behandlung wiederholt morphologische Missbildungen einer ganz bestimmten Kategorie auf. Ausserdem erwies sich Thymidin bei diesen Konzentrationen auch als mutagen zumindest in bezug auf die Auslösung geschlechtsgebundener rezessiver Letalfaktoren. Der mutagene Effekt kann durch Röntgenbestrahlung des Thymidins vor der Anwendung herabgesetzt werden. Auf die möglichen Ursachen der teratogenen und mutagenen Wirkung des Thymidins bei *D. melanogaster* wird hingewiesen.

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Reversal of the Inhibitory Effect of (2-Chloroethyl)trimethyl-ammonium Chloride on the Flowering of *Chenopodium rubrum* L. by Kinetin

In a previous paper[1] it had been shown that (2-chloroethyl)trimethylammonium chloride (CCC) ($2 \times 10^{-3} M$) delays flowering of *Chenopodium rubrum* L. and that this inhibition is completely reversed by gibberellic acid (0.1 or 0.05 mg/l). Further experiments proved that the effect of CCC may be reversed also by kinetin.

The seeds of *C. rubrum* were kindly provided to us by Prof. B. G. CUMMING, University of Western Ontario, Canada. The plants were grown on half concentrated Knop's solution under constant conditions as described previously[1]. After 4 days of cultivation under non-inductive conditions, they received 4 inductive long nights of 16 h, after which they were again transferred to continuous light. On the 13th day after emergence their developmental stage was estimated according to the length of the shoot apex or of the floral bud correlating with the degree of differentiation. CCC ($2 \times 10^{-3} M$) was added to the nutrient solution during induction, kinetin was applied to the apical bud 3 times (at 5 μl) during induction. The number of plants/variant was 30. The experiments were repeated 5 times.

As illustrated in the Table kinetin at a concentration of 1 mg/l reversed the inhibitory effect of CCC even though reversal was not always complete. When applied alone kinetin strongly inhibited flowering.

The differences between the single treatments are significant (on the 1% level) with exception of the difference between the control and the joint application of CCC and kinetin.

Effect of different treatments on length of floral bud in *Chenopodium rubrum*

Treatment	Length of floral bud (mm)
Control	0.20 ± 0.0091
CCC $2 \times 10^{-3} M$	0.15 ± 0.0048
kinetin 1 mg/l	0.12 ± 0.0073
CCC $2 \times 10^{-3} M$ + kinetin 1 mg/l	0.19 ± 0.0062

Kinetin did not affect vegetative growth. The habitus of plants treated with kinetin did not differ from that of the controls. Plants to which both CCC and kinetin was applied resembled those having received kinetin alone.

Several authors have described a reversal of the inhibitory effect of CCC brought about by growth substances other than gibberellin. The possibility of reversing the effect of CCC by kinetin has been stated by KNYPL[2] in kale seeds and by RENNERT and KNYPL[3] in tobacco callus tissue cultures. These authors suggest that CCC might affect RNA directing the synthesis of proteins.

Our results may also be interpreted by assuming that the action of CCC was beyond the Ga system. As CCC was found to lower considerably the content of endogenous gibberellin-like substances in the apical buds of *Chenopodium*[1] and as gibberellin brought about a more pronounced reversal than kinetin both gibberellin synthesis and a more general process involved in flowering of this plant might have been affected by CCC. It is also possible that a disturbance of the endogenous balance of growth substances brought about by the application of high kinetin concentrations plays a role in the described effect.

Zusammenfassung. Chlorcholinchlorid (CCC) ($2 \times 10^{-3} M$) hemmt die Blühinduktion bei der Kurztagspflanze *Chenopodium rubrum* L. Es wird gezeigt, dass diese Hemmung nicht nur durch Gibberellinsäure, sondern auch durch Kinetin (1 mg/l) aufgehoben werden kann. Eine Behandlung mit Kinetin derselben Konzentration allein (ohne vorherige Applikation von CCC) blockiert die Blühinduktion bei *C. rubrum* fast vollständig. Der Mechanismus der CCC-Wirkung auf die Blühinduktion wird diskutiert.

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