inhibited; when IAA was added after the anaerobic period more of the endogenously formed lactate was used up by the nucleus than in the controls without IAA.

From the average of the 3 experiments reported, we may conclude that 18% of the total endogenously formed lactate was used up in the aerobic period and 28% when also IAA was added. In the last column of the Table it is shown that when ATP content of fresh nuclei is taken as 100%, the resynthesis of ATP after anaerobic treatment reached a level of 69%. This percentage is in agreement with the resynthetic capacity of ATP in rat thymus nuclei as published by BETEL and KLOUWEN[6]. The addition of 0.050 m*M* iodoacetate did not influence the resynthesis of ATP very much. Higher amounts of iodoacetate than 1 m*M* gave a clear and rapid decrease of ATP content.

The possibility exists that the inhibition of respiration and ATP synthesis by higher amounts of iodoacetate as found by other investigators[11-12] is not caused by the inhibition of glyceraldehydephosphate dehydrogenase. It is known that iodoacetate is not a completely specific inhibitor, not even for compounds with sulfhydryl groups[17].

It is clear from own experiments and from those reported by other workers that glycolysis is involved in nuclear oxidative phosphorylation. A strict dependence, however, as is suggested by MCEWEN[11], cannot be considered as established, since it is possible to inhibit glycolysis by a low concentration of iodoacetate (0.050 m*M*) while oxygen uptake and ATP synthesis are hardly diminished[18].

Résumé. Les expériences présentées montrent qu'il est possible d'inhiber au maximum la glycolyse des noyaux isolés du thymus de rat, sans influencer la respiration et la synthèse d'ATP. Elles suggèrent ainsi que, dans ces noyaux, la phosphorylation oxydative ne dépend pas forcément de la glycolyse.

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Induction of Morphological Aberrations by Enzyme Inhibition in *Drosophila melanogaster*

Induction of morphological aberrations by base analogues is reported in *Drosophila* and in *Ephestia*. It was concluded that these aberrations were probably a consequence of somatic mutation following the incorporation of the analogues in DNA[1,2]. We shall report on aberrations induced in *Drosophila melanogaster* by 5-fluoro-2-deoxyuridine (FUdR) and probably caused by enzyme inhibition.

The flies were reared in $^1/_8$ l cream bottles with 33 ml of a standard food medium (1000 ml water, 19 g agar, 54 g sugar and 16 g dried yeast).

In 5 different wild stocks (Argeles, Riverside, Groningen 3, Groningen 67 and Pacific) FUdR induced the following abnormalities in high frequency: increase of scutellar and dorsocentral bristle number and incisions of the wingborder. Other aberrations which appeared in lower frequency were: 5th vein interruption, small rough eyes, leg malformations and increase of sternopleural chaetae number.

In Table I the effect of several concentrations of FUdR is shown in ♀♀ of 2 wild stocks. In this experiment at concentrations above 1 mg/l mortality increased considerably. Development was already retarded at the lower concentration. The effect on ♂♂ was similar, but the frequency of extra bristles was lower and the frequency of wing notches was higher. On 3 of the wildstocks (Argeles, Riverside and Groningen 3) comparable concentrations of 5-fluorouracil, 5-bromouracil and azauracil had no effect.

In contrast, the folic acid analogue aminopterin caused a similar syndrome of abnormalities (Table I). This is in agreement with results of SCHULTZ[3] who obtained the same effect by adding another folic acid analogue amethopterin.

It has been shown by several authors[4,5] in different organisms that FUdR blocks the synthesis of thymidine by inhibition of thymidylate synthetase. The folic acid analogues inhibit the enzyme dihydrofolic acid reductase and prevent the synthesis of tetrahydrofolic acid[6]. Tetrahydrofolic acid is a cofactor of thymidylate synthetase[7]. So, both aminopterin and FUdR affect the same step in the synthesis of thymidine. If this causes the abnormalities in *Drosophila*, it must be expected that addition of folic acid and thymidine will prevent the effect of aminopterin, and thymidine the effect of FUdR. Results of such an experiment are in agreement with this hypothesis (Table II). Preliminary results of experiments in which the flies were reared on chemically defined sterile media suggest that also folic acid deficiency causes the appearance of extra bristles.

The conclusion seems justified that FUdR causes morphological aberrations by inhibition of an enzyme (probably thymidylate synthetase) for thymidine synthesis and not by incorporation of this analogue in DNA or RNA.

It seems possible that RIZKI's results[1] can be explained in the same way. When he added 5-bromo-2-deoxyuridine (BUdR) and 5-fluorouracil (FU) separately he did not find any effect. But BUdR and FU added together caused abnormalities viz. supernumary bristles. He suggests that FU causes a thymidine deficiency, then BUdR would be incorporated in DNA and would cause somatic mutation. However, evidence obtained on mammalian cells[8] suggests the possibility that partition of BUdR could supply the 2-deoxyribose-1-phosphate necessary for conversion

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of FU in FUdR. This, then, would result in inhibition of thymidylate synthetase.

An important problem is how aminopterin and FUdR cause simultaneously extra bristles, notching of the wing-border and rough eyes. The normal differentiation of these organs depends very much on the ordered orientation and the rate of mitotic divisions[9–11]. Both aminopterin and FUdR retard mitotic divisions[12,13]. Moreover, BERTSCHMANN[14] found that nitrogen mustard, which is also a strong inhibitor of cell division[15], causes the same kind of abnormalities as aminopterin and FUdR. Therefore, it seems possible that the abnormalities caused by aminopterin and FUdR are a consequence of disturbance of the normal pattern of cell division.

Induction of morphological aberrations (often phenocopies of mutants) in *Drosophila* can be achieved by a variety of environmental manipulations, e.g. temperature shocks and all kinds of teratogenic agents[16–18]. Often the percentage of animals affected is rather low and the results are different in different stocks, both in terms of frequency and types of abnormalities. In our experiment aminopterin and FUdR show a very specific action: the same syndrome is found in wild stocks of different origin. However, this must be expected in view of the action on specific enzymes, which can only be compared with the action of mutant genes. Antimetabolites which inhibit specific enzymes are in fact the ideal phenocopying agents and will be useful tools in elucidation of the relation between differences on the genic level and their effect.

The number of scutellar and dorsocentral bristles in *Drosophila* has been used as a quantitative character in numerous selection experiments[19–22]. The genetic variability for this morphological character, revealed by the large selection responses, must be based on molecular variability viz. variability in the amount or activity of enzymes. The connection between molecular variability and genetic variability in quantitative morphological characters is a central problem in population genetics. Until now, the hope to establish this connection had to rely on a gradual increase in overlap of genes responsible for quantitative variation with genes causing isoenzymic variation[23]. If our interpretation of the action of aminopterin and FUdR by inhibition of enzymes is correct, this kind of substances can be useful for a systematic approach to this problem. When by inhibition of an enzyme a change occurs of a quantitative character, it seems probable that a change of the gene producing the enzyme concerned, can cause a similar change. Then, it will be worth while to look for isoenzymes and their relation to the quantitative character concerned[24].

Table I. The effect of different concentrations of fluorodeoxyuridine (FUdR) and aminopterin (A) in ♀♀ of *D. melanogaster*

Stock	FUdR mg/l	0.0	0.5	1.0	2.0	3.0	4.0
Pacific	No.	252	195	225	74	44	52
	sc. br. %	6.35	29.23	36.00	5.41	36.37	46.15
	mean sc. br.	4.07	4.37	4.59	4.09	4.41	4.63
	notch %	0.00	0.51	2.67	2.71	18.19	7.69
Groningen 67	No.	295	241	243	25	12	57
	sc. br. %	0.00	12.03	22.63	8.00	8.33	66.67
	mean sc. br.	4.00	4.16	4.35	4.12	4.85	5.11
	notch %	0.00	3.73	6.58	4.00	33.33	50.88
Stock	A mg/l	0.0	1.0	2.5	5.0	7.5	10.0
Pacific	No.	189	140	97	131	52	95
	sc. br. %	6.87	40.00	54.64	80.91	88.46	89.47
	mean sc. br.	4.04	4.63	4.91	6.30	7.19	6.89
	notch %	0.00	0.00	12.86	10.00	48.00	30.00

The FUdR figures are averages of 3 cultures, each stocked with 200 eggs. The aminopterin figures are averages of 3 cultures in which flies had laid an unknown number of eggs. No., number of flies scored; sc. br. %, percentage of flies with more than the normal number of 4 scutellar bristles; mean sc. br., the mean number of scutellar bristles per fly; notch %, percentage of flies with notches along the wingborder.

Table II. The effect of fluorodeoxyuridine (FUdR), aminopterin (A), thymidine (T) and folic acid (F) in ♀♀ of the Groningen 67 wildstock

	No.	sc. br. %	d. br. %	Notch %
Control	253	0.40	0.00	0.00
FUdR 1	179	1.12	1.12	1.12
FUdR 2	137	0.73	11.76	20.44
A 2	144	60.42	93.06	27.08
T 2000	198	0.00	1.52	0.00
FUdR 1 + T 1000	227	0.00	0.00	1.32
FUdR 1 + T 2000	191	0.00	0.00	2.62
FUdR 2 + T 1000	230	0.43	0.00	0.87
FUdR 2 + T 2000	197	0.00	0.51	0.51
A 2 + T 1000	206	4.85	6.80	0.00
A 2 + T 2000	231	0.43	2.60	0.00
A 2 + F 5	226	3.10	4.42	1.77
A 2 + F 100	222	0.90	0.45	0.45

Concentrations in mg/l. Figures are averages of 3 cultures. Each culture was stocked with 200 eggs. Aberrations are given as the percentage of individuals showing increase of scutellar bristles (sc. br.), increase of dorsocentral bristles (d. br.) or notches along the wingborder.

Zusammenfassung. Zusatz zum Futter von *Drosophila melanogaster* von 5-Fluoro-2-deoxyuridin oder Aminopterin induziert überzählige Skutellar- und Dorsozentralborsten sowie gekerbte Flügel. Diese Modifikationen wurden als Konsequenz von Enzymhemmung interpretiert.

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