Units of cholinesterase activity found in 100 normal adult males and 30 males suffering from cancer of the lung

ChE plasma		AChE ml of blood		AChE ml of cells		ChE whole blood	
Normal	Cancer	Normal	Cancer	Normal	Cancer	Normal	Cancer
98 ± 19	44 ± 20[a]	136 ± 14	94 ± 20[b]	285 ± 25	254 ± 29[c]	233 ± 25	167 ± 42[d]

Average and standard deviation. [a] $P<0.001$. [b] $P<0.001$. [c] $P<0.001$. [d] $P<0.001$.

Plasma. Contains: Working plasma 4.2 ml, 0.1 ml of the sample; 0.20 ml of substrate. Whole blood. Contains 1 ml of saponin, 0.1 ml of the sample, 7.5 ml of working blood, 0.40 ml of substrate. The corresponding blank contains 1 ml of saponin, 0.1 ml of the sample, 7.9 ml of distilled water. Cells. The procedure is exactly the same as for whole blood but using the corresponding sample.

In all three, immediately after adding the substrate, the spectrophotometer is read at 620 nm. Incubation for 30 min at 25 °C and the reading is repeated. The ratio between the final and initial readings is obtained and the units of pseudocholinesterase or acetylcholinesterase obtained found on the curve. To express the concentration of acetylcholinesterase per millilitre of cells, the following formula is applied:

$$\frac{\text{Value obtained in cellular curve} \times \text{hematocryt}}{100}$$

Care should be taken of the following: (1) The pH of the buffer indicator (Stock BI) must be exact. (2) The salt of acetylcholine used should be kept in a dryer to avoid hydration; once the solution has been prepared it should be kept in a refrigerator. It should be renewed every 15 days. (3) The reagents should be kept at 4–6 °C.

Results and discussion. The results obtained with this technique in normal subjects and in patients with pulmonary cancer are represented in the corresponding Table. The differences were statistically significative ($P<0.001$).

It is indispensable to fix the correct normal limits by making the statistical analysis of a sufficient number of normal subjects adding and subtracting from the mean value obtained twice the amount of standard deviations. It is also necessary to make these calculations in both men and women as the values in the latter are slightly lower than those of the males.

To use these determinations as a test for the presumptive diagnosis of neoplastic diseases, there are some circumstances that modify the values of the cholinesterase enzymes that should be kept in mind; liver insufficiency, anticholinesterase drugs, administration of ACTH or corticosteroids. The results of a positive reaction for the presumptive diagnosis of cancer should be expressed: 1 cross (+), 2 crosses (++), 3 crosses (+++) or 4 crosses (++++), according to whether the abnormal decrease is present in one or all of the different determinations.

Résumé. On décrit une méthode colorimétrique perfectionnée pour la détermination simultanée de l'activité pseudocholinestérasique, de l'acétylcholinestérase par millilitre de globules et de sang et de la cholinestérase sanguine totale, méthode actuellement employée par les auteurs pour le diagnostic présomptif du cancer. Les différences entre 100 sujets normaux et 30 malades de cancer pulmonaire ont été statistiquement significatives ($P<0.001$) dans les quatre déterminations.

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On the Dependence of Nuclear Oxidative Phosphorylation on Glycolysis in Isolated Rat Thymus Nuclei

As has been established by several authors[1–8], cell nuclei isolated from the thymus glands of calf and rat contain cytochromes and show an oxygen dependent ATP synthesis. Therefore oxidative phosphorylation is no longer a process exclusively associated to mitochondria. The cell nucleus of the thymus appears to be a complex organelle, also containing enzymes and metabolites of glycolysis and of the citric acid cycle[9–10]. MCEWEN[11] demonstrated the dependence of respiration and ATP synthesis on glycolysis. In his experiments he found a decreased respiration and ATP content when he used the glycolytic inhibitor iodoacetate (IAA), which blocks glyceraldehydephosphate dehydrogenase (1.2.1.12). From his figures it was striking that ATP content was lowered by 89%, whereas oxygen uptake was decreased by only 31%. In his experiments 1 mM IAA was used; the nuclei were isolated from calf thymus. BETEL[12] used 1.5 mM IAA

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and found, in isolated rat thymus nuclei, an inhibition of respiration and ATP synthesis of 50%.

In the present study the influence of increasing amounts of iodoacetate on glycolysis and respiration was examined. The lowest amount of iodoacetate giving maximal inhibition of glycolysis was used to investigate its effect on ATP synthesis and endogenous lactate metabolism.

Thymus nuclei were isolated essentially according to the method described by KLOUWEN and BETEL[13], based on the procedure of ALLFREY et al.[14]. The purity of the nuclear preparations was judged microscopically and by the use of marker enzymes as described by BETEL and KLOUWEN[6]. Oxygen consumption was measured by Warburg manometry. At the end of these incubations the contents of the main compartment was treated with perchloric acid to a final concentration of 3% and centrifuged at 2000 *g* for 15 min. The supernatant and the washings of the sediment were neutralized by 5 *M* K_2CO_3 and lactate was estimated by an enzymatic procedure[15]. The sediment was washed with ethanol and ether, dried, treated with 5% perchloric acid, boiled for 10 min and centrifuged for 15 min at 2000 *g*. The supernatant was used for DNA determination according to DISCHE[16].

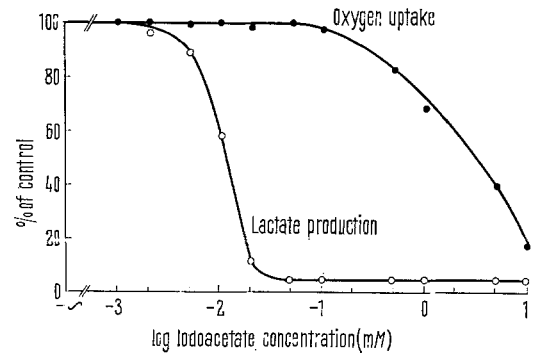
ATP synthesis and endogenous lactate content were measured in the following suspensions: fresh nuclei, nuclei which were kept under nitrogen for 15 min at 30 °C, and nuclei which were subsequently incubated for 30 min at 25 °C with air as the gas phase. These nuclear suspensions were centrifuged for 7 min at 700 *g* at 2 °C. The sediment and the supernatant were separately treated with perchloric acid. The supernatant was neutralized and assayed for lactate enzymatically[15]. This amount of lactate was considered as 'extra nuclear'. 'Intra nuclear' lactate and ATP[15] content were determined in the neutralized acid-soluble extract of the sediment. The extracted sediment was treated as mentioned above and used for the determination of DNA.

In the first group of experiments 10 m*M* glucose was used as exogenous substrate. This amount of glucose did not influence the respiration of thymus nuclei and gave a maximal lactate production at 25 °C when incubated under air for 1 h.

As can be seen in the Figure, amounts of iodoacetate above 0.001 m*M* inhibited glycolysis. An inhibition of respiration did not occur until a concentration above 0.050 m*M* was reached. A maximal inhibition of nuclear glycolysis was reached at 0.050 m*M* iodoacetate. Experiments to examine the effect of this concentration of iodoacetate on endogenous lactate and ATP content are reported in the Table.

As noted above, lactate content in the nucleus was distinguished from lactate content in the incubation medium. When fresh nuclei were kept under nitrogen for 15 min, the endogenous lactate content increased and diffused partly into the medium. Although more lactate had left the nucleus than was retained inside, the concentration outside was still very low because of the larger volume of the medium as compared with that of the nuclei. If the anaerobic suspension was incubated aerobically under air, the lactate in the nucleus decreased clearly but the concentration in the medium did not alter very much. Possibly this extranuclear concentration was too low for the lactate to re-enter the nuclei.

When iodoacetate was added before the anaerobic period, endogenous anaerobic lactate production was



The effect of increasing amounts of iodoacetate on glycolysis and respiration. The incubation medium contained: 10 m*M* glucose, 0.25 *M* mannitol, 15 m*M* NaCl, 3 m*M* $CaCl_2$, 50 m*M* *Tris* (pH 7.4) and the nuclei of about one rat thymus gland (about 0.5 mg DNA P) in a final volume of 2.5 ml. Temperature 25 °C.

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Changes in endogenous lactate and ATP content after anaerobic and subsequent aerobic treatment of rat thymus nuclei with and without 0.050 m*M* iodoacetate (IAA)

Incubation conditions	Lactate content Experiment 1 µmoles/mg DNA P intra	extra	% total[a]	Experiment 2 µmoles/mg DNA P intra	extra	% total[a]	Experiment 3 µmoles/mg DNA P intra	extra	% total[a]	Average % total	ATP content Average % total
Fresh nuclei	0.14	0.12	30	0.12	0.10	25	0.19	0.16	41	32	100
After 15′N_2	0.28	0.51	100	0.32	0.56	100	0.30	0.55	100	100	18 ± 5 (3)
After 15′N_2 + IAA	0.13	0.22	44	0.14	0.24	43	0.17	0.19	42	43	13 ± 4 (3)
After 15′N_2; 30′ air	0.10	0.54	81	0.15	0.59	84	0.11	0.58	81	82	69 ± 4 (3)
After 15′N_2 + IAA; 30′ air	0.09	0.22	39	0.10	0.25	40	0.10	0.20	35	38	60 ± 3 (3)
After 15′N_2; 30′ air + IAA	0.07	0.51	73	0.09	0.55	73	0.07	0.53	70	72	70 ± 4 (3)

Anaerobic incubations were performed under nitrogen for 15 min at 30 °C without shaking (15′N_2). Subsequent aerobic incubations were carried out in a metabolic shaker with air as the gas phase for 30 min at 25 °C (30′ air). The incubation medium was the same as mentioned under the figure. [a] Total lactate content is summarized from intra- and extranuclear lactate content and is expressed as percentage of total lactate content after 15′N_2.

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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[18] The author is indebted to Dr. H. M. KLOUWEN for introducing the isolation procedure of thymus nuclei in our laboratory and to Miss SJOUKJE HAASJES for technical assistance.

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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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