(up to 2%) of transformed cells appeared in some, while no such cells were seen in the remainder of the leucocyte cultures which were either derived from unsensitized donors or contained dinitrophenylated erythrocytes or serum proteins.

By contrast, the cultures of leucocytes from sensitized guinea-pigs, directly exposed to FDNB, contained 8–22% transformed cells. While this observation strongly suggests that FDNB has a specific action on sensitive cells, it indicates that neither erythrocyte nor serum protein conjugates act as transforming agents. Consequently, it seems most probable that FDNB reacts directly with the leucocytes. Since 97–100% of the leucocytes in the cultures were lymphocytes, it seems virtually certain that the latter contributed most of the reacting cells. Moreover, the high reactivity of the halogen-substituted dinitrobenzens[5] renders it probable that conjugation occurs at the cell surface. These considerations lead us to suggest that the first essential reaction in the induction of transformation in our system is the union of FDNB, a relatively simple chemical substance, with the components of the cytoplasmic membrane of the lymphocyte[6,7].

The effect of FDNB on lymphocyte transformation

Preparation	No. of guinea-pigs tested	Percentage of transformed cells[a]
Lymphocyte conjugates (sensitized donors)	11	8–22
Lymphocyte conjugates (unsensitized donors)	6	0
Red cell conjugates	11	0–2
Serum conjugates	11	0–2

[a] In cultures 5 days old.

Résumé. Les lymphocytes du sang périphérique des cobayes sensibilisés au 1-chloro-2,4-dinitrobenzène ont été conjugués avec 1-fluoro-2,4-dinitrobenzène et ensuite cultivés in vitro pendant 5 jours, à partir du moment où on a observé en culture de 8–22% de cellules transformées.

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Action of Some Compounds on the Adenosine Triphosphate Pool of *Streptococcus faecalis*

In a previous report[1], the action of various compounds on the metabolic swelling of protoplasts[2–4] and on the glycolytic activity of both whole cells and protoplasts of *Streptococcus faecalis* was described. Since the effect of the majority of the compounds tested on the swelling of protoplasts did not appear to be related to their action on the glycolytic activity, the present investigation was designed to test the action of these compounds on the adenosine triphosphate (ATP) pool of whole cells of *S. faecalis*.

Material and methods. Aqueous solutions (20 µl/ml of final suspension medium) were used for glucose ($11\times10^{-3}M$), 2,4-dinitrophenol (DNP) ($1\times10^{-3}M$), arsenate, sodium salt (ARS) ($20\times10^{-3}M$), dicumarol (DIC) ($50\times10^{-6}M$), and sodium azide ($10\times10^{-3}M$). Ethanol (95°) solutions (1 µl/ml of final suspension medium) were used for gramicidin (GRAM) ($22\times10^{-6}M$), oligomycin (OLIG) ($15\times10^{-6}M$), rutamycin (RUT) ($100\times10^{-6}M$), and valinomycin (VAL) ($0.35\times10^{-6}M$). The above figures express the molarities in the final suspension medium.

S. faecalis ATCC 9790 was grown as reported before[1]. The cells were harvested by centrifugation, washed 3 times with redistilled water and twice with $0.075M$ potassium phosphate, pH 6.2, resuspended in a convenient volume of $0.075M$ potassium phosphate pH 7.2 to give a protein content between 1.0–1.3 mg/ml, and placed in a water bath at 38 °C. The compound being tested was added first, followed by glucose 15 min later; with GRAM the order was inverted for the reasons already stated[1]. Samples were collected at regular intervals. To each milliliter of the sample 50 µl of 70% perchloric acid were added. The mixture was allowed to stand for 30 min at room temperature, neutralized with KOH and chilled in ice. The supernatants collected after centrifugation at 3000 r.p.m. for 15 min, diluted as needed, were used for the assay of ATP using the firefly luminescence technique[5]. Firefly extract was prepared from fireflies desiccated tails, according to FRANZEN and BINKLEY[6]. Lactic acid was assayed using the Sigma Chemical Co. set, since in *S. faecalis* all the lactic acid produced from glucose is L(+)[7].

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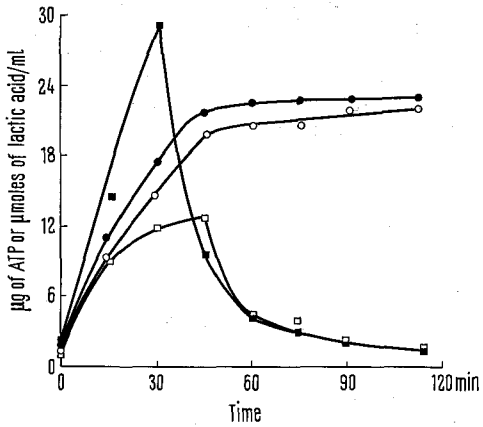
Results and discussion. Application of FORREST[8] formulations to our results for the ATP pool in the controls (where only glucose was added), yielded mean values of 0.043/min for the decay constant (k), of 0.527 µg/min for the input constant (b), and of 3.511 for the ratio of the number of total µmoles of ATP put into the pool against the number of µmoles of glucose added (T_{ATP}/G). This last value is in good agreement with the maximal theoretical value for the production of ATP from glucose[9]. Our observed values when plotted against $1-e^{-kt}$ seem to agree fairly well with the linear hypothesis for the input of ATP into the pool; further, the expected values calculated on the basis of linear kinetics are in very good agreement with the observed values.

The Table represents the action of the various compounds on the values of k, b, and T_{ATP}/G. The main effect of ARS was a decrease of the ratio T_{ATP}/G, which was expected since ARS is known to reduce the efficiency of glycolysis. A decrease of the ratio T_{ATP}/G and an increased rate of decay of the ATP in the pool were observed with GRAM. However, the lower T_{ATP}/G ratio does not necessarily mean that less energy is produced. It is possible that some of the energy produced might be used before entering the pool as ATP. In washed cells suspended in K_2HPO_4, the most likely consumptive activity would be an inflow of phosphate, potassium or both and/or an outflow of some intracellular material. A GRAM effect on the K^+–H^+ exchange across mitochondrial membranes was described[10] with a net result of an increased concentration of K^+ in the intracellular medium. On another hand K^+ was reported to stimulate glycolytic activity either by raising the intracellular pH[11] or by stimulation of phosphoenolpyruvate transferase[12] or phosphofrutokinase[13]. If GRAM also increases the intracellular concentration of K^+ on *S. faecalis*, the observed stimulation of glycolytic activity of this bacteria in the presence of GRAM[1] can be better understood. Azide mainly increased the rate of decay of the ATP in the pool and the total ATP input into the pool, without the rate of input being greatly affected. This does not agree with the hypothesis of SPIEGELMAN, KAMEN and SUSSMAN[14] according to which this compound reduced the energy production during glucose metabolism. Both DNP and DIC stimulated the rate of ATP input into the pool; while the first, also stimulating the rate of decay of the ATP in the pool, did not seem to affect significantly the total amount of ATP put into the pool, DIC nearly doubled it. This total ATP input in the presence of DIC corresponds by itself to an ATP/glucose ratio greater than the theoretical maximum. Possible explanations for this value include: a glucose metabolic pathway other than the Embden-Meyerhof's; and availability by the action of DIC of some cellular material to energy yielding reactions. These and any other explanations should take into consideration the fact that nearly 2 moles of lactic acid are recovered per each mole of added glucose (Figure)[15,16].

Influence of various compounds on the input constant (b), and decay constant (k) for the ATP pool of glucose fermenting cells of *S. faecalis*, and on the ratio of the calculated total µmoles of ATP put into the pool against the µmoles of glucose added (T_{ATP}/G)

	b	k	T_{ATP}/G
2,4-Dinitrophenol $1\times10^{-3}M$	1.50	2.11	1.16
Sodium azide $10\times10^{-3}M$	0.81	1.75	1.40
Dicumarol $50\times10^{-6}M$	2.27	1.14	1.92
Sodium arsenate $20\times10^{-3}M$	1.14	1.28	0.59
Gramicidin $22\times10^{-6}M$	0.90	1.41	0.52
Oligomycin $15\times10^{-6}M$	0.92	0.73	0.85
Valinomycin $0.35\times10^{-6}M$	1.21	0.96	1.16
Rutamycin $100\times10^{-6}M$	0.98	0.84	0.87

The values for the control are taken as unity.



ATP pool (■—■) and lactic acid production (●—●) from glucose in *S. faecalis* in the presence of dicumarol $50\times10^{-6}M$. □—□ control for the ATP pool and ○—○ control for lactic acid production. Glucose $11\times10^{-3}M$ added at time 0. Dicumarol added 15 min before glucose.

Résumé. Les auteurs étudient le contenu en ATP chez le *Streptococcus faecalis*, après l'addition de glucose et en présence de diverses substances. Les effets les plus évidents ont été ceux du dicumarol augmentant la valeur de la constante d'entrée et la quantité de l'ATP; du 2,4-dinitrophénol augmentant les 2 constantes (d'entrée et de décomposition); de l'arsénate réduisant la quantité de l'ATP; de l'azide augmentant la constante de décomposition; et de la gramicidine qui augmente non seulement la constante de décomposition mais réduit aussi la quantité de l'ATP dans le «pool».

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