glycogen phosphorylation in muscle and the production of lactic acid both in muscle and blood. Thus, the animals were anaesthetized with Nembutal 50 mg/kg and a sample was either homogenized with 6% perchloric acid for lactic acid determination or ground with sand in an ice-cold solution containing 3.8% sodium fluoride, 0.08*M* EDTA pH 7.0 (2 vol. of solution/g of muscle) for phosphorolysis assay. The pH 7.0 extract was centrifuged and 0.5 ml of the supernatant were incubated for 30 min at 37 °C with 0.5 ml of 1% glycogen and aliquots of 0.25 ml of the mixture were removed at different times and added to 4 ml of 10% trichloracetic acid. After centrifugation, glycogen phosphorolysis was determined assaying inorganic phosphorus in an aliquot of the supernatant, according to LOHMAN's and JENDRASSIK's method[5]. Aliquots of blood were also taken for lactic acid determination.

After removal of muscle sample and blood, the anaesthetized animals were injected with DNP 2.5 mg/100 g and were kept for 10 min to permit the drug action. New samples of muscle and blood were taken and treated as above.

Table II shows that when the amount of lactic acid was high in blood and muscle, the glycogen phosphorolysis was inhibited in muscle. These parallel events strongly support our previous observations[1,2].

Resumen. La fosforilase *b* del músculo esquelético es inhibida por el 2,4-dinitrofenol, de acuerdo con nuestras observaciones anteriores. La fosforilase *a* no es inhibida por la droga.

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Stimulation of Lymphocyte Transformation by 1-Fluoro-2,4-Dinitrobenzene

The addition of specific antigens to lymphocytes in vitro stimulates the appearance of transformed cells[1,2]. However, it is uncertain whether the transformation follows direct contact between the antigen and the lymphocyte or is the outcome of an intermediate step involving either antigen and some other cell such as the macrophage, or an antigen-antibody reaction.

We are investigating the action of antigen in hypersensitivity using 1-fluoro-2,4-dinitrobenzene (FDNB) because, in addition to being a well-established inducer of contact hypersensitivity, this substance reacts rapidly with proteins to form stable compounds[3]. The identification of the cellular localization of these compounds and their chemical composition may uncover the steps in the development of hypersensitivity, and in particular of delayed hypersensitivity.

We have found that the addition of FDNB to cultures of lymphocytes, obtained from guinea-pigs sensitized to the dinitrophenyl moiety, is followed by the appearance of transformed cells.

The test animals were outbred female, albino guinea-pigs weighing 600–800 g. Sensitization was induced by injection into the nuchal skin of 40 µg 1-chloro-2,4-dinitrobenzene dissolved in distilled water, i.e. a modification of the method of CHASE[4]. 11 days later, the animals were bled under aseptic conditions by severing the jugular vein and collecting blood from each animal into separate, sterile bottles containing glass beads, 3–5 mm in diameter. After gentle shaking for 5–10 min, the defibrinated blood was transferred to fresh bottles and to it was added 1% Methocel (USP Grade, Dow Chemicals Co.) in 0.2*M* phosphate buffer, pH 7.2 (0.3 ml/ml blood). After mixing, the blood was allowed to settle for 45 min at 37 °C. Sedimentation resulted in the separation of a layer of leucocyte-enriched serum. The upper $^2/_3$ of this leucocyte layer was centrifuged for 5 min at 200 *g*, the supernatant removed and the cells washed twice with Hanks solution, with centrifugation between washings. The resultant preparation of leucocytes consisted of 97–100% lymphocytes. The proportion of erythrocytes to leucocytes in the preparation was about 1:1.

Erythrocytes and serum were obtained from the residual defibrinated and sedimented blood by centrifugation for 10 min at 200 *g*. The serum was harvested, the remaining leucocytes discarded and the red cells washed twice with Hanks solution.

From the blood of each of 11 sensitized animals, 3 sets of duplicate leucocyte cultures were then prepared. The first set contained leucocytes conjugated with FDNB; the second, conjugated erythrocytes and unconjugated leucocytes; the third, unconjugated leucocytes and serum to which FDNB had been added.

The white and red cells were separately conjugated with FDNB by suspension for 30 min in sodium phosphate buffer 0.2*M* pH 8.5, adjusted to contain 10 µg FDNB per $1.0–1.3 \times 10^6$ cells/ml. After incubation, the cells were centrifuged for 5 min at 200 *g*, the supernatant discarded and the cells washed thrice with Hanks solution. The leucocytes were then suspended (approximately 10^6 cells) in a culture medium consisting of 2 ml Medium 199 (Commonwealth Serum Laboratories, Melbourne) and 1 ml autologous serum. Cultures of leucocytes from unsensitized guinea-pigs were similarly prepared. The conjugated erythrocytes were mixed with an approximately equal number of unconjugated, autologous leucocytes before being suspended in culture medium.

Conjugated serum proteins were prepared by adding sodium phosphate buffer, 0.2*M*, pH 8.5, containing 10 µg/ml FDNB to each ml of serum and incubating the mixture for 30 min at 37 °C. This mixture was then added to Medium 199 in a proportion of 1:2 and used to suspend unconjugated, autologous leucocytes.

The Table summarizes the percentage of transformed cells appearing in the various cultures. Small numbers

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(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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[7] We wish to thank Dr. R. LAM PO TANG, Prof. D. L. WILHELM and Dr. A. W. J. LYKKE for advice.

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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