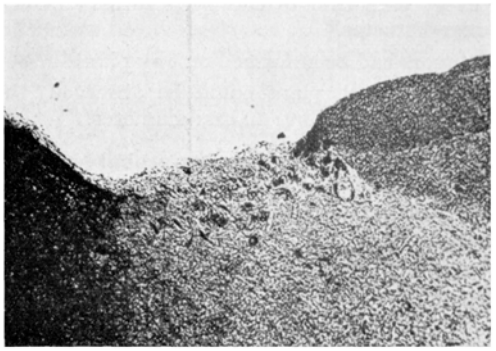

Fig. 1.—The activity of acetylcholinesterase in the nucleus supraopticus of a control rat. × 72.

Fig. 2.—The activity of acetylcholinesterase in the nucleus supraopticus of a serotonin treated rat. × 72.

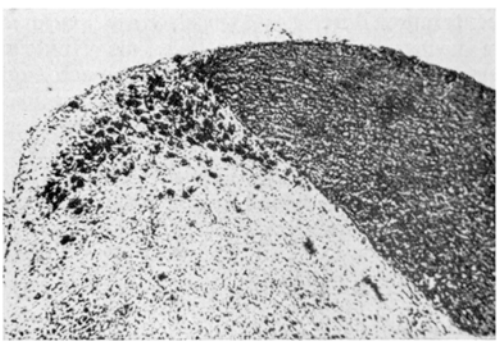

Fig. 3.—The activity of acid phosphatase in the nucleus supraopticus of a control rat. × 72.

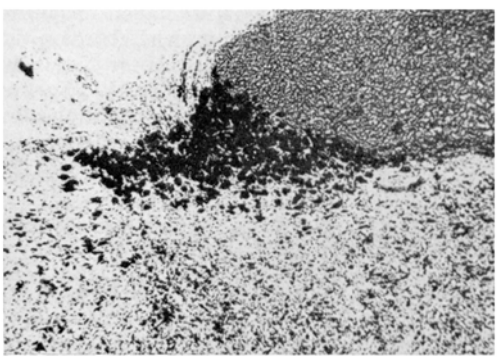

Fig. 4.—The activity of acid phosphatase in the nucleus supraopticus of a serotonin treated rat. × 72.

On the other hand, it is known that acid phosphatase is involved in the protein synthesis mechanism. The fact that serotonin increases the activity of just these enzymes thus tends to strengthen the hypothesis that serotonin stimulates the activity of the nuclei under consideration[5,6]. The activity of the respiratory enzyme, succinic dehydrogenase, is normally fairly slight in the n.s.o. and n.p.v.; therefore it has been assumed that aerobic metabolism has no great significance in the activity of the cells[15]. Also the result obtained in this work can be considered an indication in this direction.

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Zusammenfassung

Es wurde histochemisch festgestellt, dass Serotonin eine mässige Steigerung der Acetylcholinesterase- und sauren Phosphataseaktivität des Nucleus supraopticus und Nucleus paraventricularis bewirkt. Es wird an eine Aktivierung des N. supraopticus und N. paraventricularis durch Serotonin gedacht.

[15] N. Shimizu, N. Morikawa, and Y. Ishi, J. comp. Neurol. *108*, 1 (1957).

* Aided by a grant from the Sigrid Jusèlius Stiftelse.

The Role of Nucleic Acids in Crown-Gall Tumor Induction

In a previous paper[1], the preparation of a tumor inducing principle TIP which was germ-free was described. These experiments, first made on *Pelargonium zonale*, have been extended to other plants. Finally it was decided to use *Datura stramonium*, a plant which reacted well and speedily to inoculations of virulent strains of *Agrobacterium tumefaciens* (Smith and Townsend), the causal agent of Crown-Gall. The germ-free induction of Crown-Gall tumors was achieved by incubating the bacteria for a two-day period at 27°C together with a juice expressed from plants, wounded 48 h beforehand. A sterile filtrate of this mixture was capable of inducing typical tumors on *Datura*. Furthermore it was demonstrated that TIP was not obtained by adding a deoxyribonuclease to the mixture before incubation.

These results have now been confirmed, and in addition the deoxyribonuclease was found to have no effect if added to the mixture after incubation. The fact that this enzyme can inhibit the formation of TIP *in vitro* would emphasize the importance of the role of nucleic acids in Crown-Gall tumor induction. Such results show that the active principle of the bacterium is closely related to the nucleic acids, but it is also clear that the nucleic acids are not identical with TIP and are not alone responsible for the tumor induction. Manil *et al.*[2] have studied this problem and have prepared different fractions of nucleic acids from a mass culture of the organism. The proliferations on plants after inoculation with these preparations were very limited and, as the authors state, not typical. It may be

[1] P. Manigault, A. Commandon, and P. Slizewics, Ann. Inst. Pasteur *91*, 114 (1956).

[2] P. Manil, L. Delcambe, and J. Fourneau, Bull. Acad. R. Belg. Cl. Sci. [5] *41*, 259 (1955).

that the extraction methods[3] used did not produce a native nucleic acid.

The extraction methods used by us were different to those of MANIL *et al.*[2]. The nucleic acid we obtained was transferred together with specific 'wound juice'. This gave tumors which were morphologically, histologically, and physiologically identical with tumors induced by the microbe alone.

The strain 5.6 of *Agrobacterium tumefaciens* was first cultivated for 48 h on a nutrient agar at room temperature, transferred to Fernbach flasks and finally to aerated culture bottles which contained 10 l of the following liquid culture medium: KH_2PO_4 2 g, Na_2HPO_4 1·5 g, KNO_3 0·25 g, K_2SO_4 0·5 g, $(NH_4)_2HPO_4$ 1 g, $MgSO_4 \cdot 7H_2O$ 0·25 g, Rochelle salt 2 g, dextrose 40 g, distilled water ad 1000 cm³. The pH was adjusted with KOH to 7·6–8·0. Incubation was for 48 h at 27°C. The bacteria were collected with a Sharpless type centrifuge at 40,000 r.p.m. and washed three times in a Tris-buffer 0,1 *M* at pH 8·0. The organisms were suspended in a solution of 18·6 g Versene (Komplexon III) in sodium-potassium-phosphate-buffer 0,01 *M* at pH 7·4 to which was added 9 g of NaCl in 1 l. The bacterial suspension was then frozen at −60°C and defrozen at room temperature several times to enhance the breakage of the cell walls. Total lysis was realised in a 10% sodium lauryl sulfonate solution at pH 8·0 at 37°C. The very high amount of proteins required frequent deproteinisation with the Sevag method: the liquid was shaken with a mixture of chloroform-amyl alcohol 9:1 and centrifuged until no more protein could be collected in the zone between the two phases. Precipitation of all the nucleic acid was with 1·3 volumes of ethanol at 95%. It was then purified by solution in 0·9% NaCl and reprecipitation several times with ethanol. The final precipitate was dissolved in sterile NaCl 0·9% and stored at 0°C. The 'wound juice' was obtained from 4–6 weeks old plants of *Datura stramonium*. The upper internodes of the stem were slit spirally with a razor blade. 48 h later these internodes were 'homogenized' in a blendor together with solid CO_2. After filtering by pressing through a cheeze cloth, 3 cm³ of 'wound juice' were easily obtained from each plant. This sap was clarified in a MSE-centrifuge at 5000 r.p.m. for 15 min. The supernatant was now lyophilized. For each experiment, the dried material was reconstituted with distilled water to the original volume and sterilized by passing through a Seitz-filter. The sterility of each sample was tested.

Three successive experiments were made with 5–7 replications in each. Inoculation of the 4-weeks old plants of *Datura stramonium* was by means of special pipettes. These were closed at the end, but had a hole of similar size at the side. They were pushed into and then fastened to the 'wound' made on removal of one of the upper leaves. Through them 0·3 cm³ of liquid was infiltrated in 24–36 h into each plant. After the liquid had been taken up, the pipettes were removed.

The test series were as follows:

(A) A 48-h old culture on nutrient agar of strain 5.6 of *Agrobacterium tumefaciens* in 0·9% NaCl.

(B) The sterile nucleic acid fraction described alone.

(C) An equal mixture of the nucleic acid fraction and 'wound juice', the nucleic acid being added to the 'wound juice' just before the infiltration of the plant.

The results after a 50-day period were:

(A) In every case tumors had been formed from 10 to 30 mm diameter.

(B) No tumors had been formed on any plant.

(C) Tumors on each plant could be observed, the size varying between 5 and 20 mm diameter.

Photographs of these tumors are probably being published[4].

The following conclusions have been drawn from these results: The function of *Agrobacterium tumefaciens* in the induction of Crown-Gall tumor is due to the nucleic acids and very probably to the deoxyribonucleic acid. However TIP can not be identical with the nucleic acids of *Agrobacterium*, because their infiltration gave rise to no tumors. We suggest that an important protein fraction of the 'wound juice' is modified by the nucleic acid and this becomes the TIP. This is the first time—so far as we know—that a *Crown-Gall tumor* has been *induced without* the presence of the *microbe* in any one of the different phases.

It is intended to determine in the future which nucleic acid is active and to fractionate the important principle of the 'wound juice' as well as to elucidate the reaction mechanism of the induction.

We want to acknowledge our thanks to Dr. P. SCHAEFFER (Institut Pasteur de Paris) who has been assisting us in developing the nucleic acid extraction method. The second author is grateful for the help and accommodation provided by the Pasteur Institute.

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Institute Pasteur, Paris, and Institute for special Botany of the E.T.H., Zürich, September 11, 1958.

Zusammenfassung

Die Arbeit befasst sich mit der Induzierung von Pflanzentumoren durch das *Agrobacterium tumefaciens*. Es konnte nachgewiesen werden, dass die Nukleinsäurefraktion aus dem Bacterium imstande ist, nach Zugabe zu einem Homogenisat von verletzten *Datura-stramonium*-Pflanzen ein Tumor auslösendes Prinzip aufzubauen. Eine derartige (sterile) Fraktion ist in der Lage, echte Crown-Gall-Tumoren zu induzieren, deren Identität mit den bakteriell erzeugten Tumoren morphologisch, histologisch und physiologisch erwiesen ist.

[3] A. MIRSKY and A. POLLISTER, Proc. nat. Acad. Sci., Wash. *28*, 344 (1942).

[4] P. MANIGAULT and CH. STOLL, Ann. Inst. Pasteur, in press (1958).

Zur Biosynthese des Ubichinons

LOWE, MORTON und HARRISON[1] haben im Jahre 1953 gefunden, dass im Unverseifbaren der Leber von Vitamin-A-Mangelratten Substanzen unbekannter Natur angereichert werden, die auf Grund typischer UV.-Spektren erkannt werden können. Die weitere Bearbeitung führte zur Reindarstellung einer Komponente, die später als Ubichinon bezeichnet wurde[2]. Am 18. Juli 1958 wurde in Shef-

[1] J. S. LOWE, R. A. MORTON und R. G. HARRISON, Nature *172*, 716 (1953).

[2] G. N. FESTENSTEIN, F. W. HEATON, J. S. LOWE und R. A. MORTON, Biochem. J. *59*, 558 (1955). – N. F. CUNNINGHAM, J. S. LOWE, L. MERVYN, R. A. MORTON und J. VERNON, Proc. biochem. Soc. *60*, xviii (1955). – F. W. HEATON, J. S. LOWE und R. A. MORTON, J. chem. Soc. *1956*, 4094. – J. S. LOWE, R. A. MORTON und J. VERNON, Biochem. J. *67*, 228 (1957). – R. A. MORTON, G. M. WILSON, J. S. LOWE und W. M. F. LEAT, Chem. & Ind. *1957*, 1649.