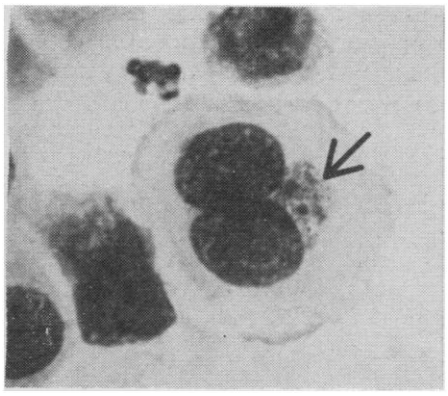

Fig. 1. Inclusion body in epithelial cell of patient's conjunctiva.

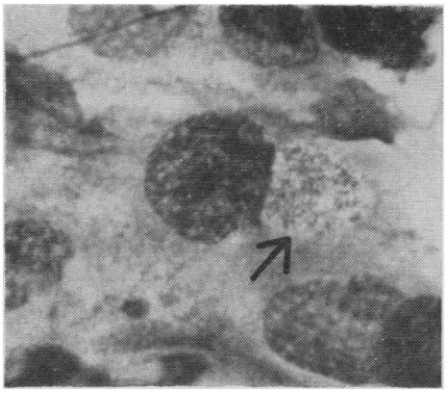

Fig. 2. Inclusion body in conjunctival epithelial cell of *Macacus cynomolgus* inoculated three days earlier with sixth-egg-passage virus (Bour).

His physician prescribed 1-percent chloramphenicol eyedrops, which he instilled for the next 26 days, with some symptomatic improvement. On 12 February a similar conjunctivitis developed in the right eye, with marked photophobia. Neomycin-polymyxin eyedrops were administered for 4 days. On 16 February the inflammation in the left eye suddenly increased markedly, and gross keratitis appeared. Clinical examination on 17 February revealed bilateral papillary hypertrophy, with buried follicles of the upper tarsi, microscopic pannus and corneal infiltrates, and palpable preauricular lymph nodes. Scrapings from the upper tarsal conjunctivas showed many inclusions typical of trachoma (Fig. 1). Bacterial cultures were negative. The patient was treated with topical tetracycline and oral methylsulfapyridazine (500 mg daily) from 19 Feb. to 15 Apr. 1959, with gradual disappearance of all clinical and microscopic signs of trachoma. At present he appears healed.

Conjunctival scrapings were collected on 17 and 19 February in broth-saline containing streptomycin (1000 μg/ml) (*1*, *2*). After 1 hour at 4°C they were injected into the yolk sac of 6- and 8-day-old embryonated eggs. In this first egg passage there was no mortality, and no elementary bodies were seen in smears of the yolk sac stained in accordance with Macchiavello or Giemsa techniques. Elementary bodies were seen in five of six eggs of the second passage sacrificed on days 9 and 10 after inoculation. In the third egg passage all embryos died between the fourth and the eighth day after inoculation, and elementary bodies were seen in profusion. At present this virus strain (Bour) in the seventh egg passage has an egg LD_{50} of $10^{-4.5}$. The elementary bodies conform in size and staining properties with those grown by others from trachoma patients (*1–3*). Four monkeys (*Macaca cynomolgus*) were inoculated in one eye with a 20-percent yolk-sac suspension of sixth-passage virus and in the other eye, with normal yolk sac. Two to six days later they all developed follicular conjunctivitis, and conjunctival scrapings contained many typical inclusion bodies (Fig. 2).

The patient's serum, drawn on 19 February, fixed complement in a 1:64 dilution with psittacosis or Lygranum antigens. Antigens prepared from a yolk-sac pool of virus (Bour) fixed complement with antisera to psittacosis virus (*4*).

This appears to be the first isolation of an elementary-body virus of the psittacosis-lymphogranuloma group ("trachoma virus") from a typical case of trachoma arising in the United States. Many intriguing questions are now under study, including the relationship of this virus (Bour) to inclusion blennorrhea and to the elementary-body viruses isolated from trachoma in other parts of the world, the toxin production and pathogenetic potential of this agent, and its biological and epidemiological characteristics (*5*).

L. HANNA
P. THYGESON
E. JAWETZ

Francis I. Proctor Foundation, University of California Medical School, San Francisco

C. DAWSON

Epidemic Intelligence Service, Communicable Disease Center, U.S. Public Health Service

References and Notes

1. F. F. T'ang, H. L. Chang, Y. T. Huang, K. C. Wang, *Chinese Med. J.* **75**, 429 (1957).
2. L. H. Collier and J. Sowa, *Lancet* **I**, 993 (1958).
3. E. S. Murray, J. C. Snyder, S. D. Bell, *Proc. Intern. Congr. Trop. Med. and Malaria, 6th. Congr., Lisbon* (1958).
4. The antisera was provided through the courtesy of B. Eddie.
5. This work was supported by grants from the National Institutes of Health (B-604), the Burroughs Wellcome Fund, and the Research Committee of the University of California.

6 July 1959

Growth Inhibitor in Immature Soybean Seeds and 2,4-D-Sprayed Soybean Seedlings

Abstract. A naturally occurring inhibitor of seed germination has been isolated by ion-exchange chromatography from soybean seeds and seedlings. The inhibitor was present in large amounts in immature seeds and in seedlings sprayed with 2,4-D. The inhibitor acted as an "uncoupler" when applied to soybean root tips or mitochondria.

Our concurrent studies on the inhibition of germination in immature soybean seeds (Galitz) and on the inhibition of growth in soybean seedlings by 2,4-dichlorophenoxyacetic acid (2,4-D) (Key) indicated that a common compound was responsible for the two inhibitions. In the studies of growth inhibitions that resulted from spraying seedings with $5 \times 10^{-4}M$ 2,4-D, soybean hypocotyls were extracted with $0.6M$ cold perchloric acid. The extracts were cleared of perchlorate and chromatographed on Dowex-1-formate for separation of nucleotide components (*1*). One elution peak (designated *C* in this report) increased about twofold in seedlings which had been sprayed with 2,4-D 24 hours before extraction. Compound *C* eluted from the ion-exchange column between adenosine monophosphate and guanosine monophosphate when a gradient of formic acid was the eluent. There was a positive correlation between the concentration of compound *C* and the growth inhibition induced by 2,4-D. Further investigation showed that compound *C* was present in mature soybean seeds and young seedlings but declined rapidly during germination in the absence of 2,4-D treatment.

Galitz (*2*) reported the presence of a water-soluble inhibitor in immature soybean seeds. These seeds could be induced to germinate by leaching with water for 2 to 4 hours. The leachate obtained from these seeds would retard elongation of radicles excised from mature seeds. Immature seeds were therefore investigated for the presence of *C*.

Figure 1 shows the elution chromatogram of perchloric acid extracts of immature soybean seeds collected 25 to 30 days after flowering. The dry weight of such seeds was approximately 25 percent of the dry weight of mature seeds. Compound *C* accounted for over 50 percent of the 260-mμ absorbing material. Compound *D* appeared to be derived from *C* during extraction, for, if the perchloric acid extracts were held overnight at 2° to 4°C, there was a loss of *C* and an increase in *D*. The amount of *C* in immature seeds was 2 to 3 times as great as in mature seeds; on a dry-weight basis the concentration

was 8 to 10 times higher. Immature seeds which were leached in water for 3 hours lost over half of the *C* initially present. Compound *C* was the only fraction obtained from the perchloric acid extracts which was an effective growth inhibitor.

For inhibition studies, compound *C* was isolated from immature soybean seeds or frozen green peas (a readily available source of material) by ion-exchange chromatography, as illustrated in Fig. 1, then lyophilized to remove the formic acid. The dried residue was dissolved in water and adjusted to *p*H 5.0 with KOH. Concentrations are expressed in terms of optical density at 260 mμ. One "O.D. unit" is that amount of *C* which will give unit optical density in 1 ml of solution with 1-cm cuvettes in the Beckman DU spectrophotometer.

Table 1 gives data for the effect of compound *C* on seed germination, on root respiration and phosphate accumulation, and on oxidative phosphorylation by mitochondria isolated from soybean hypocotyls. Mature soybean seeds were soaked in the indicated concentrations of *C* for 4 hours and then planted in moist vermiculite. Germination of treated seeds was delayed 24 hours as compared with germination of controls soaked in water. After 48 hours, 60 to 90 percent of the treated seeds had germinated. The mean length of the embryonic axis of the seeds that germinated is given in Table 1. Similar responses to compound *C* were obtained with mature corn seeds.

Table 1. Effects of compound *C* on germination of soybean seeds and on the respiration and phosphate uptake of soybean root tips and isolated soybean hypocotyl mitochondria.

Germination of soybean seed			
Units of *C* per 4 ml of water	0	1	10
Length of plant (in cm) (after 48 hours)	4.5	2.5	2.0
*Activity of 1-cm soybean root tips**			
Units of *C* per 2.5 ml of buffer	0	5	25
Respiration, initial 15 minutes	780	830	1670
Respiration, after 3 hours	753	809	943
PO_4 uptake, after 3 hours	0.31	0.26	0.21
Activity of soybean mitochondria†			
Units of *C* per 2.5 ml of buffer	0	1	15
$Qo_2(N)$	917	885	702
P/O	2.69	2.27	2.08
P/N	221	179	130

* Root tips were placed in Warburg vessels in $10^{-3}M$ potassium phosphate (*p*H 5.0) labeled with P^{32}. Respiration rate is given as microliters of O_2 per hour per gram (fresh weight); phosphate uptake is given as micromoles of PO_4 absorbed per gram (fresh water).

† Mitochondria were isolated and activity was determined over a 30-minute period with α-ketoglutarate as the substrate (*8*). $Qo_2(N)$ is given in microliters of O_2 per hour per milligram of N; P/O is given in micromoles of P esterified per microatom of O_2; P/N is given in micromoles of P esterified per hour per milligram of N.

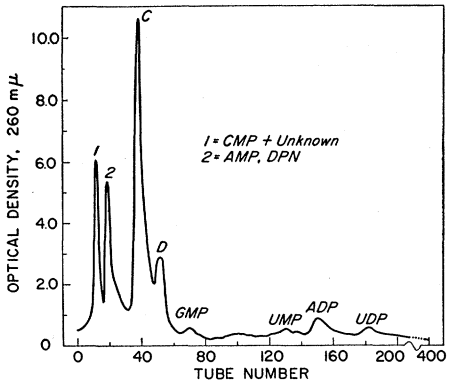

Fig. 1. Elution spectrum of soluble nucleotides from immature soybean seeds. Seeds were homogenized in 0.6*M* $HClO_4$ and centrifuged, and the perchlorate was removed from the extract as the K salt. The extract was placed on Dowex-1 (formate) and eluted with a gradient of formic acid-ammonium formate (*1*). ADP, AMP, adenosine di- and monophosphate; CMP, cytidine monophosphate; DPN, diphosphopyridine nucleotide; GMP, guanosine monophosphate; UDP, UMP, uridine di- and monophosphate.

The application of 25 O.D. units of compound *C* to root tips caused the respiration rate to increase markedly in the initial 15 minutes; this increase was followed by a gradual decline to rates comparable to control levels after 1 hour. Table 1 shows respiration rates and the amount of orthophosphate accumulated by the tissue in 3 hours. The net result in increased respiration and in decreased phosphate accumulation was similar to that produced by the uncoupling agent, 2,4-dinitrophenol.

Further evidence that compound *C* acts as an uncoupler was given in the experiments with mitochondria. Phosphate esterification per unit N was inhibited. Oxidation of α-ketoglutarate was generally depressed, as indicated in Table 1, although in occasional experiments a transitory increase in respiration was noted.

It will be noted in Fig. 1 that no triphosphate nucleotides were obtained from immature seeds. Extracts of hydrated mature seeds contained appreciable amounts of triphosphate nucleotides, particularly adenosine triphosphate. This finding and the evidence that compound *C* is an uncoupler of oxidative phosphorylation (Table 1), suggest that *C* acts as a growth inhibitor, at least in part, through inhibition of high-energy phosphate production.

The concept that endogenous inhibitors control seed germination is widely held (*3*), and Evenari (*4*) has listed over 100 species from which germination inhibitors have been obtained. Compound *C* may be an important inhibitor of germination in immature seeds. Woodford *et al.* (*5*) and van Overbeek *et al.* (*6*) have suggested that the growth inhibitions caused by high auxin concentrations may result from the accumulation of growth inhibitors. The experiments reported here (*7*) indicate that the inhibitory effects of 2,4-D applied to soybean seedlings may be the result of the accumulation of *C*.

Work on the chemical identification of compound *C* is in progress. Studies are also being made of the distribution of this compound in various species of immature seeds and of its relationship to seed germination.

JOE L. KEY
DONALD S. GALITZ
Department of Agronomy, University of Illinois, Urbana

References and Notes

1. J. H. Cherry, thesis, Univ. of Illinois (1959); R. B. Hurlbert, H. Schmitz, A. F. Brumm, V. R. Potter, *J. Biol. Chem.* **209**, 23 (1954).
2. D. S. Galitz, *Plant Physiol. Suppl.* **33**, xxxi (1958).
3. E. H. Toole, S. B. Hendricks, H. A. Borthwick, V. K. Toole, *Ann. Rev. Plant Physiol.* **7**, 299 (1956).
4. M. Evenari, *Botan. Rev.* **15**, 153 (1949).
5. E. K. Woodford, K. Holly, C. C. McCready, *Ann. Rev. Plant Physiol.* **9**, 311 (1958).
6. J. van Overbeek, R. Blandeau, V. Horne, *Plant Physiol.* **26**, 687 (1951).

7. This investigation was supported in part by grants from Regional Research Project NCM-23, U.S. Department of Agriculture, and from the National Science Foundation. We wish also to acknowledge the use of facilities of the U.S. Regional Soybean Laboratory, and to thank R. W. Howell and J. B. Hanson for advice during the course of the investigations.

8. H. A. Lund, A. E. Vatter, J. B. Hanson, *J. Biophys. Biochem. Cytol.* **4**, 87 (1958).

15 June 1959

Discrimination Learning

Abstract. Eight rats were run through discrimination training sessions in which responses in the dark were not reinforced whereas the first response after the onset of a light was reinforced. The procedure generated orderly learning and latency data for the individual animal. The latency distributions are adequately described by a simple mathematical formulation.

The present study was designed, first, to determine the extent to which orderly discrimination learning curves for individual animals could be simply obtained and, second, to test the adequacy of a mathematical formulation developed by Mueller (*1*) for describing latency data.

The subjects were eight naive, adult, male albino rats which had been deprived of water for 22½ hours at the start of each experimental session. The apparatus consisted of a response chamber through one wall of which a