vision. From these preliminary results it appeared that shock intensity (as measured in milliamperes) was not the determining factor in the postoperative performance. In the free situation the animals behaved exactly as described by both Sherrington and Twitchell. While the monkey was running, the limb was held in a semiflexed position, hand and fingers hanging loosely. The deafferented limb was not used for climbing, and occasional attempts to use the limb for defense always ended in failure. In the conditioning situation, however, the flexion responses were fairly consistent and occurred without obviously associated head and neck movements.

The questions of whether these responses were centrally directed or whether the animals learned to make use of cues provided by intact afferents in other parts of the body are presently being investigated.

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14 July 1958

Restoration of Tryptophan Synthetase Activity in Escherichia coli by Suppressor Mutations

Immunological studies with mutants of *Neurospora crassa* defective in the ability to form the enzyme tryptophan synthetase (TSase) (indole + L serine → L-tryptophan) have shown that certain of these strains form large amounts of a protein, designated CRM, which is immunologically similar to TSase (*1*). One mutant, td_1, allelic by genetic criteria with the other mutant strains (*2*), was found to lack CRM (*1*). On the basis of these observations it was tentatively concluded that CRM represents an altered form of TSase and that one gene controls the formation of TSase and CRM (*3*, *4*).

Extensive mutational studies carried out with strain td_1 have shown that this strain does yield tryptophan-independent cultures and that these cultures invariably result from reversions at the td locus rather than suppressor mutations (mutations of different genes reversing the effect of the primary mutation) (*5*). Furthermore, other tests demonstrated that td_1 does not respond to suppressor genes which affect one or more of other mutants lacking TSase (*2*). Since lack of suppressibility appeared to be associated with inability to form CRM in this strain, the possibility was considered that only strains capable of forming a slightly altered TSase are capable of responding to suppressor genes (*3*).

Mutants of *Escherichia coli* lacking TSase also fall into two categories with respect to CRM formation; one group forms a protein which is immunologically similar to TSase, while the other group does not (*6*). The latter group appears to be comparable to the td_1 type in *Neurospora*. In an effort to examine more thoroughly the question of whether mutants lacking CRM are suppressible, one *Escherichia coli* stock which lacks CRM, strain T-41, was selected for further study (*7*). Cell suspensions of this strain were irradiated with ultraviolet light and plated on a medium lacking tryptophan in a search for suppressor-type mutations. Many small and large trytophan-independent colonies appeared on the plates after 3 days of incubation; several of each type were picked and purified by streaking on a medium lacking tryptophan. Three small- and three large-colony types were selected and examined further in accumulation tests. The three small-colony types accumulated indoleglycerol as does T-41, the mutant they were derived from, while the three large-colony types did not accumulate detectable amounts of any compound related to an intermediate in the tryptophan pathway.

The six selected cultures were also examined in transduction tests (*8*) to determine whether suppression or reversion was responsible for their tryptophan independence. Phage grown on each of the strains listed was used to transduce a cysteine-requiring stock (the cysteine and T-41 genes are closely linked) to cysteine independence, and the treated cells were plated on a medium containing tryptophan. The resulting colonies were then tested for tryptophan dependence. If typtophan-dependent colonies were obtained, the original stock must have carried both the T-41 mutant gene and a suppressor gene. If no mutants were recovered, the original stock probably was a revertant. The three small-colony types yielded typical T-41-like trytophan-dependent colonies in these tests, indicating that suppression was responsible for their ability to grow in the absence of tryptophan. The three large-colony types did not yield mutants, and thus they appear to represent reversions at the T-41 mutant locus. The presence of suppressor genes in the presumed suppressed stocks was confirmed by transducing the suppressor genes from these stocks into strain T-41.

Table 1. Characteristics of the various strains examined.

Strain	Type	Accumulation	Specific activity of TSase	TSase neutralized by 0.02 ml of antiserum
T41	Mutant	Indoleglycerol	0	—
T41-R2	Suppressed mutant	Indoleglycerol	1.5	1.08
T41-R3	Suppressed mutant	Indoleglycerol	1.5	1.38
T41-R4	Suppressed mutant	Indoleglycerol	1.0	1.28
T41-R6	Revertant	None	2.7	1.41
T41-R7	Revertant	None	3.3	1.3
T41-R8	Revertant	None	3.0	1.15
K-12	Wild type	None	2.8	1.24

Enzyme and immunological studies were performed with the six cultures and are summarized in Table 1. It can be seen that extracts of all six strains exhibit TSase activity, while the strain they were derived from, T-41, does not. It can also be seen that the TSase formed by the six cultures is normal in the sense that approximately equivalent amounts are neutralized by TSase antiserum. The three suppressed mutants appear to form somewhat less TSase than the revertants and the wild-type strain.

These findings indicate that suppressor mutations can restore the ability to form an enzymatically and antigenically active protein to a mutant which lacks CRM.

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6 May 1958

Occurrence of Substances with Juvenile Hormone Activity in Adrenal Cortex of Vertebrates

No effects of vertebrate hormones upon insect growth have ever been demonstrated (*1*). During the past several years we have examined the effects of hundreds of growth factors including a series of vertebrate hormones (*2*), on the development of insects. Particular care was taken to inject the hormones at critical stages in the insect's life history when a hormonal effect on development would presumably be easiest to detect.

The most startling results were obtained with extracts of beef adrenal cortex (*3*). When such extracts were injected into pupae of the Polyphemus silkworm (*Antheraea polyphemus*), the pupae precociously molted into strange creatures which were intermediate between pupa and adult. These intermediates displayed large patches of pupal cuticle and retained such juvenile characters as pupal antennae and immature genitalia (Fig. 1). Indeed, they were indistinguishable from pupae that were injected with extracts of juvenile hormone prepared from insects (*4*) and from others into which active corpora allata were implanted. The experiments have been repeated several times with the same results. Aqueous extracts of the adrenal cortex mimic in detail the action of the juvenile hormone of insects. To our knowledge this is the first instance of a substance extracted from a vertebrate that has a specific morphogenetic effect on an insect, or indeed, on any invertebrate.

It is somewhat surprising that the first chemical extracted from a vertebrate to influence invertebrate growth should resemble the juvenile hormone, for this molecule appears to have no functional counterpart in the vertebrates. In insects it promotes larval development and prevents maturation. A similar agent is not known for vertebrates.

The active principle in the beef extracts has not yet been isolated, but we do know that it is not identical with any of more than 50 cortical components and their derivatives which we have tested so far. Nor can the effects of the cortical extract be duplicated by any of several hundred hormones, vitamins, metabolites, antimetabolites, and enzymes that have been tested (*1, 5*). In short, we are dealing with what appears to be a unique and specific group of substances with juvenile hormone activity, rather than nonspecific substances such as the numerous unrelated chemicals that activate crustacean chromatophores.

Since the cortical extract is rich in steroids, it is tempting to suggest that the active principle and the juvenile hormone itself are steroids. Such a conclusion is consistent with all that we presently know about the chemistry of the juvenile hormone (*4, 6*). In this connection it is noteworthy that Knowles, Carlisle, and Butler have reported that the ovarian-inhibiting hormone of prawns and bees may be a steroid and that they mention briefly that the effect of this hormone can be copied by administering the synthetic sex hormone, methyltestosterone (*7*). If the juvenile hormone and the ovarian-inhibiting hormone prove indeed to be steroids, then this important class of biologically active compounds assumes a central role in the humoral control of growth in invertebrates as well as vertebrates. Rather than being a recent innovation of the vertebrates, steroid hormones may prove to have a far more ancient lineage. Whether the active principle in the cortical extract is similar chemically to the juvenile hormone or merely acts in a similar way is being investigated. The possibility that the extract turns on the corpora allata of the injected pupae is also being studied.

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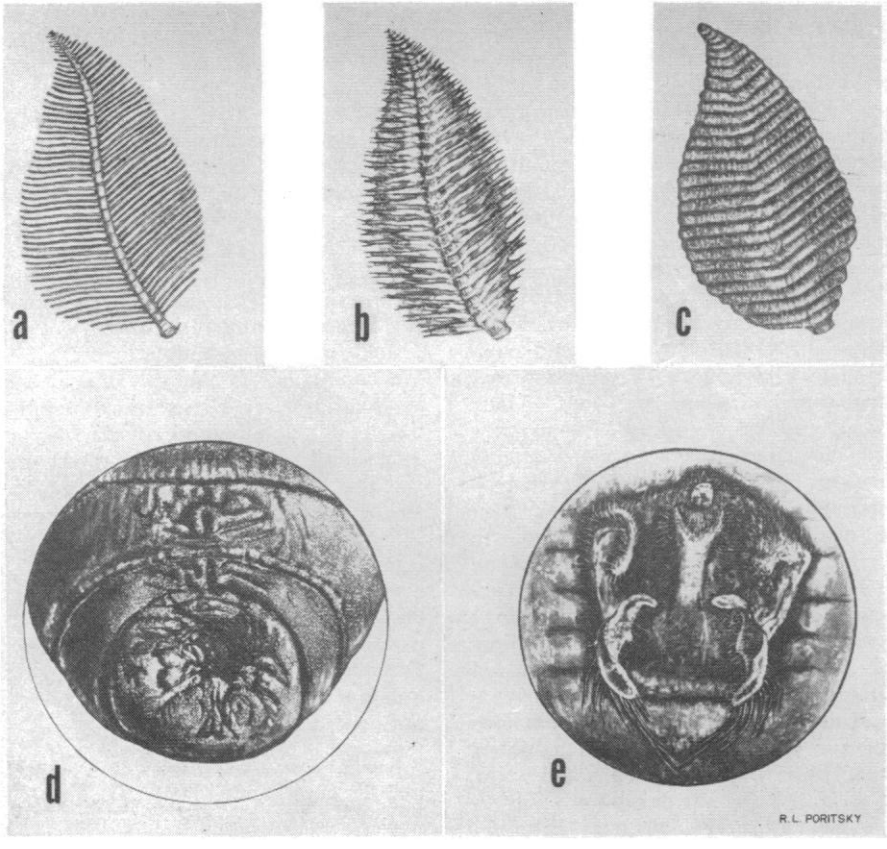

Fig. 1. (*A*) Antenna of an adult Polyphemus moth. (*B*) Antenna of an "intermediate" Polyphemus moth produced by injecting a pupa with adrenal cortical extract prior to initiation of adult development. (*C*) Antenna of a Polyphemus pupa. (*D*) Genitalia of an "intermediate" Polyphemus moth produced by injecting adrenal cortical extract into a pupa prior to initiation of adult development. (*E*) Genitalia of an adult Polyphemus moth.

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24 April 1958