of counts per minute per milliliter of intracellular water to counts per minute per milliliter of incubation medium. The activity of intracellular water was determined by subtracting the radioactivity in the extracellular volume from that in the total tissue water. Total tissue water and extracellular volume were determined under incubation conditions similar to those used in the AIB-1-C^{14} experiments. Total tissue water was obtained by drying diaphragms to constant weight at 105°C, and extracellular volume was obtained by isotope dilution, employing sucrose uniformly labeled with C^{14}. Sucrose-C^{14} distribution reached an apparent equilibrium after approximately 15 minutes of incubation, but increased about 2 percent between 1 and 2 hours of incubation. After 2 hours of incubation, the sucrose-C^{14} space of normal tissue was found to be slightly higher (19.3 percent) than that of muscle of hypophysectomized rats (17.4 percent). The addition of growth hormone preparations to the medium did not markedly alter the magnitude of the sucrose-C^{14} space. In some experiments, amino acid nitrogen determinations (*7*) were made on aliquots of the medium following incubation to ascertain the amount of amino acid nitrogen released by the tissue.

The results are given in Figs. 1 and 2, in which the penetration of AIB-1-C^{14} into muscle is plotted against incubation time. AIB-1-C^{14} enters muscle cells of hypophysectomized rats at a lower-than-normal rate (Fig. 1). Adding either simian or bovine growth hormone preparations to the medium at a concentration of 25 μg/ml greatly enhanced the uptake of AIB-1-C^{14} by the diaphragms of hypophysectomized rats. The fact that both simian and bovine growth hormones stimulated entry of AIB-1-C^{14} into islolated diaphragm was of particular interest, since only simian growth hormone was found to consistently stimulate leucine-2-C^{14} incorporation into diaphragm protein when added in vitro (*2*). When the concentration of the hormone preparations was reduced to 2.5 μg/ml of medium there was still a doubling in the rate of AIB-1-C^{14} penetration (Fig. 2). However, a further 10-fold reduction in hormone concentration nearly eliminated the stimulatory effect. The greater rate of AIB-1-C^{14} penetration in the hypophysectomized controls in the second series of experiments (Fig. 2) may be related to the shorter time (14 days) that they were hypophysectomized.

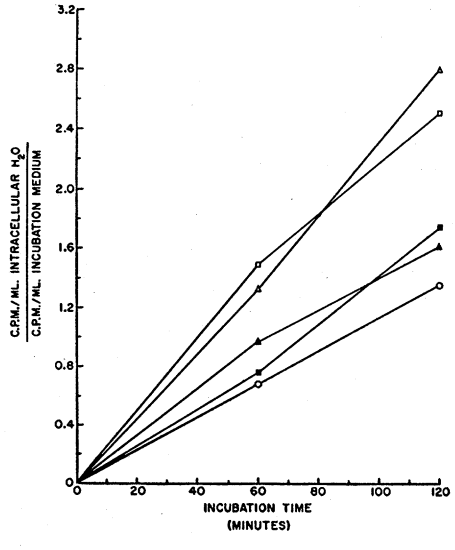

Fig. 2. Effect of low in vitro concentrations of growth harmone preparations on the penetration of AIB-1-C^{14} into "intact" diaphragms of hypophysectomized rats. ○, hypophysectomized control; △, simian growth hormone (2.5 μg/ml); ▲, simian growth hormone (0.25 μg/ml); □, bovine growth hormone (2.5 μg/ml); ■, bovine growth hormone (0.25 μg/ml). Each point represents one observation.

Measurements of the amounts of amino acid nitrogen released into the medium during these experiments indicated that on a tissue-weight basis, the "intact" diaphragm preparation of a normal rat released somewhat more amino acid nitrogen into the medium than did that of a hypophysectomized animal, confirming the observations of Kline (*8*). The addition of growth hormone preparations to the medium had no effect on the rate of release of amino acid nitrogen. Since the amounts of tissue used in most experiments were approximately equivalent, the concentration of amino acid nitrogen in the medium at any time during the incubation was roughly comparable between experimentals and controls, differing only rarely by as much as 5 μg/ml of medium. Thus, the hormonally induced differences in AIB-1-C^{14} transport noted in these experiments are probably not ascribable to changes in the concentration of nonlabeled, endogenously produced amino acids.

The experiments presented here support the hypothesis that pituitary growth hormone plays a role in the regulation of amino acid transport into muscle cells. Should growth hormone be found to stimulate the transport of natural amino acids as well, then the increase in amino acid incorporation into protein produced by this hormone might well be due, in part at least, to an initial increase in the availability of amino acid (*9*).

J. L. KOSTYO*
J. HOTCHKISS†
E. KNOBIL
Department of Physiology, Harvard Medical School, Boston, Massachusetts

References and Notes

1. J. L. Kostyo and E. Knobil, *Federation Proc.* **18,** 84 (1959).
2. ———, *Endocrinology* **65,** 395 (1959).
3. ———, *ibid.* **65,** 525 (1959).
4. M. W. Noall, T. R. Riggs, L. M. Walker, H. N. Christensen, *Science* **126,** 1002 (1957).
5. D. M. Kipnis and M. W. Noall, *Biochim. et Biophys. Acta* **28,** 226 (1958).
6. D. M. Kipnis and C. F. Cori, *J. Biol. Chem.* **224,** 681 (1957).
7. E. G. Frame, J. A. Russell, A. E. Wilhelmi, *ibid.* **149,** 255 (1943); J. A. Russell, *ibid.* **156,** 467 (1944).
8. D. L. Kline, *Endocrinology* **45,** 596 (1949).
9. We wish to express our gratitude to A. E. Wilhelmi for generously supplying the simian growth hormone used in these experiments. The bovine growth hormone was a gift of the endocrinology study section of the National Institutes of Health. This study was supported by a grant from the American Cancer Society (P-139A).
* National Research fellow in the medical sciences. Present address: Department of Medicine, Duke University Medical Center, Durham, N.C.
† Predoctoral research fellow, National Institutes of Health.

27 July 1959

Stimulation of Striga asiatica (Witchweed) Seed Germination by 6-Substituted Purines

Abstract. Kinetin [6-(2-furfuryl)aminopurine] and certain other 6-substituted aminopurines stimulated germination of seed of *Striga asiatica* (L.) Kuntze. Optimum concentration for most active compounds was in the range of 5 to 25 mg/lit. Derivatives which showed high activity possessed an adenine nucleus with a phenyl, benzyl, phenethyl, or furfuryl radical substituted on the amino group.

Striga asiatica (L.) Kuntze, an angiospermous root parasite indigenous to several tropical and subtropical areas of the Eastern Hemisphere, was discovered in the coastal plain section of North Carolina and South Carolina in 1956. This species threatens warm-season gramineous crops in the infested regions. Consequently, attention is being focused for the first time in the United States on problems associated with the growth, development, and control of this particular plant parasite.

Germination of *Striga* seed depends upon a substance (or substances) secreted by the roots of host and certain other plant species (*1*). The natural germination stimulant of *Striga* spp. has not been identified. However, a sugar, D-xylulose, was reported to be very effective in promoting germination of seed of *S. hermonthica* (Del.) Benth. but did not appear to be a constituent of the root exudate of *Sorghum vulgare* (*2*). In conjunction with efforts to isolate and characterize the chemical stimulant, or stimulants, in root exudates of seedlings of corn, *Zea mays* L., various organic and inorganic compounds were tested for their ability to induce seed germination. In preliminary tests 6-(2-furfuryl)aminopurine

(kinetin) (*3*) was found to stimulate germination. Subsequently, the germination-stimulation potentials of other 6-substituted purines (*4*) were examined.

Striga seed used in this study were collected during the summer of 1957 and stored at room temperature. The seed are very small and even after imbibing water measure only approximately 0.15 by 0.20 mm. Prior to being used in germination tests, the seed were preconditioned for 15 to 20 days on moist filter paper in the dark at 23° to 24°C. The requirements for preconditioning in order to obtain maximum germination in laboratory studies have been previously established (*2*) and were reconfirmed in the study reported here. In the germination tests a small piece (about 4 by 7 mm) of moist filter paper holding 50 to 100 seed was blotted of excess moisture and floated on 0.2 ml of the solution being tested. This solution was contained in the depression or well of a spot test plate. The entire plate was covered by a thin sheet of polyethylene and sealed around each well with mineral oil. The plate containing the seed was then placed in the dark in a germinator at 33° ± 1°C. After 24 hours, germination counts were made under 30× magnification and the results were expressed as percentages of germination. Seed were considered to have germinated when the hypocotyl had emerged from the seed coat and had attained a length one-half that of the seed.

A standard germination stimulant and distilled water were incorporated in all tests as controls. A solution obtained by growing the roots of about 35 corn seedlings in 400 ml of aerated distilled water for 5 to 10 days in the dark served as the standard stimulant. When applied to preconditioned seed this solution consistently produced 75 to 85 percent germination within 24 hours. Germination did not increase appreciably beyond this period. The seed seem to have no requirement for red light, and germination was slightly inhibited if the seed were exposed to incandescent light during germination. Germination of seed in distilled-water controls did not occur in these studies. The purine analogs were tested at concentrations of 50, 25, 10, 5, 1, 0.5, and 0.1 mg/lit.

A stock solution containing 50 mg/lit. of distilled water was initially prepared for each compound. Adjustment of the *p*H to approximately 11.2 with NaOH was required to dissolve most of the compounds. Then the *p*H was readjusted to approximately 6.5 with HCl. The results obtained with control solutions which received comparable adjustments in *p*H did not differ from those obtained with unadjusted controls.

Table 1. Effect of various 6-substituted purines on the germination of *Striga asiatica* seed.

Compound	Mean germination* (%) at indicated concentration			Mean germination for 3 rates (%)
	25 mg/lit.	10 mg/lit.	5 mg/lit.	
6-Phenylaminopurine	39	81	73	64
6-(2-Thenyl)aminopurine	54	52	63	56
6-(2-Phenethyl)aminopurine	48	75	38	54
6-Benzylaminopurine	12	70	52	45
6-(2-Furfuryl)aminopurine (kinetin)	24	62	27	38
Benzaldehyde-6-purinylhydrazone	62	17	7	29
6-(*o*-Chlorobenzyl)aminopurine	1	21	32	18
6-Hexylaminopurine	1	6	42	16
6-(*p*-Chlorobenzyl)aminopurine	1	32	9	14
6-(Tetrahydrofurfuryl)aminopurine	20	3	1	8
6-(Furfuryl)-6-(methyl)aminopurine	8	3	0	4
6-Benzylthiopurine	0	6	1	2
None (water blank control)				0
None (standard stimulant control)				81

* Average of two replications.

Maximum germination for each compound was obtained at the concentrations shown (Table 1) except for 6-hexylaminopurine and benzaldehyde-6-purinylhydrazone, which produced 45 and 89 percent germination at concentrations of 1 mg and 50 mg/lit., respectively. Most of the active compounds appeared to become inhibitory at concentrations above 10 mg/lit. For comparative purposes, the compounds are listed in Table 1 in an order based on an average germination value obtained by averaging the germination measured at concentrations of 5, 10, and 25 mg/lit. This is an arbitrary ranking and does not necessarily give an accurate indication of the germination-stimulating capacity of the compounds. The following compounds, not listed in Table 1, were inactive in this study: purine (free base); adenine; 6-(1-piperazinyl)purine; 5-nitro-2-furaldehyde-6-purinylhydrazone; 6-(hydroxyethyl)aminopurine; 6-bis(hydroxyethyl)aminopurine; (6-purinylthio)acetic acid; 6-(3-dimethylaminopropyl)aminopurine; and 6-(2,2-dimethylhydrazino) purine.

The highly active compounds were 6-substituted aminopurines in which the substituent group consisted of a phenyl, benzyl, phenethyl, or furfuryl radical. Activity was decreased when the benzene ring was chlorinated, the furfuryl ring was saturated, or the remaining amino hydrogen was replaced by a methyl group. Replacement of the oxygen in the furfuryl moiety by sulfur produced a compound which was even more active than kinetin. Among the compounds tested which contained aliphatic groups, only 6-hexylaminopurine was active. It was much less active than the compounds having a ring for the substituent group. The 6-benzylthiopurine was only slightly active in stimulating germination in *Striga* seed. However, it is very active in promoting germination in lettuce seed (*5*). Consequently, structural specificity of the 6-substituted purines seems to be somewhat more exacting for germination in *Striga* seed than for germination in lettuce seed. From a consideration of the kinetin analogs evaluated in this study, it appears that the structural requirements for stimulation of germination in *Striga* seed correspond closely to those for high cell-division-stimulating activity in the tobacco callus growth test (*6*).

Results of these studies suggest that in the stimulation of germination in *Striga* seed the 6-substituted aminopurines may participate in reactions governing germination not activated by light (*5, 7*).

Differences were noted between hypocotyls of seed stimulated to germinate by the 6-substituted purines and those in which germination was promoted by the natural stimulant present in the corn-root exudate. When the latter was used, the hypocotyls were long and slender. Elongation of the hypocotyls continued for 4 to 5 days, and the hypocotyls reached a length of 2 to 3 mm. After about 6 days the hypocotyls became flaccid, and the cells became discolored and eventually died. When purine derivatives were used at threshold concentrations, the hypocotyls had the same appearance as those treated with the natural stimulant. At all higher concentrations, however, the hypocotyls consisted of a bulbous mass of small cells. Root-hair-like protuberances were usually observed growing from epidermal cells. Within 4 to 5 days the hypocotyls began to elongate from each of the bulbous structures, and the cotyledons enlarged and emerged from the seed coats. Elongation of the shoot apex beyond the two first leaf or scale primordia was observed in some of the treatments. So far as we know this is the first time development of this type has been reported for *Striga* seedlings that

have not made haustorial connections with the host roots. The slowly developing seedlings remained alive for several weeks in the initial purine solutions. Seedlings stimulated by, and left in contact with, the natural stimulant solution did not develop enlarged cotyledons or undergo elongation of the shoot apex (*8*).

A. D. WORSHAM
Department of Field Crops, North Carolina State College, Raleigh
D. E. MORELAND
Agricultural Research Service, U.S. Department of Agriculture
G. C. KLINGMAN
Department of Field Crops, North Carolina State College

References and Notes

1. R. Brown and M. Edwards, *Ann. Botany* **8**, 131 (1944); C. Fuller, *Agr. J. Union S. Africa* **1**, 684 (1911); H. H. W. Pearson, *ibid.* **3**, 651 (1912); A. R. Saunders, *Union S. Africa Dept. Agr. Sci. Bull.* **128**, (1933); K. B. Vallance, *Ann. Botany* **14**, 347 (1950).
2. R. Brown *et al., Proc. Roy. Soc. (London)* **B136**, 1 (1949); ———, *Biochem. J.* **50**, 596 (1952); A. R. Lansdown, *Univ. of Cambridge Abstr. Diss.* (1953–54), p. 234; A. G. Long, *ibid.* (1952–53), p. 202; G. J. Tyler, *ibid.*, p. 213.
3. The kinetin used in this study was purchased from Nutritional Biochemicals Corp., Cleveland, O.
4. The other 6-substituted purines were generously supplied by Charles G. Skinner of the University of Texas, Austin, and by Parke, Davis and Co., Detroit, Mich.
5. C. G. Skinner and W. Shive, *Arch. Biochem. Biophys.* **65**, 567 (1956); *Plant Physiol.* **32**, 117 (1957).
6. F. M. Strong, *Topics in Microbial Chemistry* (Wiley, New York, 1958).
7. C. O. Miller, *Plant Physiol.* **31**, 318 (1956); *ibid.* **33**, 115 (1958).
8. These studies were cooperative investigations of the North Carolina Agricultural Experiment Station and the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture. This report is published with the approval of the North Carolina Agricultural Experiment Station as paper No. 1066.

30 July 1959

New Permian Insects Discovered in Kansas and Oklahoma

Abstract. The Midco insect bed of Oklahoma and a newly discovered insect bed above this were traced across Kay County, Okla., into Sumner County, Kan. As a result, a greater time span is available for study of insect evolution during the mid-continent Permian, and the exact stratigraphic correlation of the Wellington of Oklahoma and Kansas can now be demonstrated. Four insect orders have thus far been identified from the new insect bed: Protodonata, Odonata, Protoperlaria, and Ephemeroptera. Numerous new species and higher categories are included in the collections from the two insect beds.

In 1939 the famous Midco insect bed (Permian, Leonardian, Wellington formation) of Noble County, Okla., was discovered by G. O. Raasch (*1*) and subsequently explored by Raasch and F. M. Carpenter (*2*). Slightly northwest of a locality in southern Kay County where Raasch had previously reported no insects, Tasch, aided by his assistant, Bernard Shaffer, found insects in the Midco bed. In addition, some 8 ft above the Midco, a new insect bed was discovered (NW-SW, sec. 31, T 25 N, R 1 W).

The Midco insect bed and the new insect occurrence above it were both traced to northern Kay County (NE-NW and also SE-NE, sec. 23, T 28 N, R 28 W), where an excellently preserved insect fauna was found. At this locality algal beds occur respectively below and above the two insect beds. Equivalent algal beds were traced to Sumner County, Kan. (SE-SW, sec. 11, T 35 S, R 1 W). Insects were found associated with the upper algal bed.

This is the first stratigraphically related correlation of the Oklahoma and Kansas Wellington formation. As a result, dozens of fossil conchostracan beds that Tasch found in the Oklahoma Wellington can be related to those discovered in Kansas. This, in turn, provides the necessary stratigraphic basis for study of evolutionary changes in Permian conchostracans.

Previous work by Tasch in Kansas (*3*) established that there were two distinct insect beds: the well-known Carlton insect bed of Dunbar in Dickinson County and one below it in Marion, Harvey, and Sedgwick counties. The Midco insect bed and the newly discovered insect bed above it are stratigraphically above (that is, geologically younger than) the two insect beds of Kansas. Thus, four distinct insect beds are now known for the mid-continent Wellington formation.

Fossil Permian insects belonging to the following orders and families have been identified by Zimmerman, who is doing the insect systematics for this project.

Southern Kay County, Okla. Upper insect bed, 8 ft above the Midco: Protodonata; Odonata, Protozygoptera, Kennedyidae, *Kennedya* sp.; Protoperlaria, Lemmatophoridae.

Northern Kay County, Okla. Upper insect bed, 9.9 ft above the Midco: Ephemeroptera. Mideo: (extinct orders) Protodonata, Megasecoptera, Protelytroptera, Protoperlaria, Protorthoptera; (living orders) Ephemeroptera, Odonata, Blattaria, Corrodentia, Homoptera, Neuroptera, Mecoptera.

South Haven, Sumner County, Kan. Upper algal-insect bed, 9.3 ft above the Midco equivalent: Megasecoptera.

These findings are of unusual interest. Our knowledge of insect speciation and evolutionary trends during the American Permian had previously been limited to data from two beds: Midco and Carlton. Extension of the vertical range of Leonardian insects—above the Midco and below the Carlton—enlarges the geologic time span through which they may now be studied (*4*).

PAUL TASCH
Department of Geology, University of Wichita, Wichita, Kansas
JAMES R. ZIMMERMAN
Department of Biology, Indiana Central College, Indianapolis

References and Notes

1. G. O. Raasch, thesis, University of Wisconsin (1946).
2. F. M. Carpenter, *Proc. Am. Acad. Arts Sci.* **76**, 25 (1947).
3. P. Tasch, "Stratigraphic position of the Wellington conchostracan-bearing beds in Kansas," *Abstr. AAPG-SEPM Program, Dallas meeting* (1959), p. 60.
4. This research was supported by National Science Foundation grants Nos. G-7320 and G-4150.

31 August 1959

All-Female Strains of the Teleost Fishes of the Genus Poeciliopsis

Abstract. In addition to the viviparous fish *Mollienesia formosa*, two other species of poeciliids have recently been found to produce only female offspring. The young of these females, however, unlike those of *M. formosa*, inherit characteristics from any one of the several species of males used in experimental matings.

Self-perpetuating populations of unisexual vertebrates have been experimentally demonstrated only among the viviparous fishes of the New World family Poeciliidae—of which the guppy

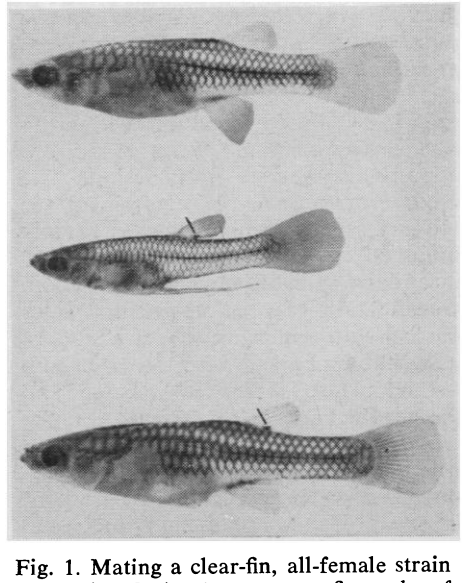

Fig. 1. Mating a clear-fin, all-female strain of species C (top) to a spot-fin male of species F (middle) results in spot-fin, all-female offspring (bottom); this demonstrates that, unlike the finding for *Mollienesia formosa*, characters of the male are transmitted to the all-female hybrids.