we calculated C, 78.55; H, 10.99; we found C, 78.48; H, 10.99.

A sample of soyasapogenol B was prepared in our laboratory according to the method given by Ochiai *et al.* It had a melting point of 257° to 258°C and a mixed melting point with the alfalfa genin I of 255° to 256°C. Infrared data (*11, 12*) indicated closely related structures for the two materials (Fig. 1). It is therefore suggested that the neutral saponins of alfalfa are of the same type as the neutral saponins of soybean in that both contain a triterpene nucleus in the aglucon portion (Fig. 2).

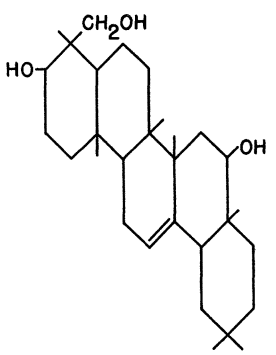

Fig. 2. The structure of soyasapogenol B, as given by Meyer *et al.* (*10*), appeared to be similar to the alfalfa genin, mp 258° to 259°C.

Both the purified alfalfa and soybean saponins inhibited the growth of chicks while their genins did not (*13*). At least part of the growth-depressing effect of uncooked soybean meal may be due, therefore, to the soyasapogenols which on cooking hydrolyze to nontoxic genins. If this cooking process is too mild, incomplete hydrolysis of the soyasapogenols could occur. Such meals might cause bloat in ruminants and would explain the occurrence of bloat in cattle fed with untoasted solvent-extracted soybean meal. On the other hand, the dehydrating conditions are too mild to hydrolyze alfalfa saponin.

References and Notes

1. D. E. Bowman, *Proc. Soc. Exptl. Biol. Med.* **57,** 139 (1944).
2. W. E. Ham and R. M. Sandstedt, *J. Biol. Chem.* **154,** 505 (1944).
3. J. W. Hayward, H. Steenbock, and G. Bohstedt. *J. Nutrition* **12,** 275 (1936).
4. S. Lepkovsky *et al., Poultry Sci.* **29,** 208 (1950).
5. D. Peterson, *J. Biol. Chem.* **183,** 647 (1950).
6. I. L. Lindahl *et al., Science* **119,** 157 (1954).
7. Funds for partial support of these studies were made available by Armour and Co., Chicago, Ill. This work is also reported in a thesis presented by George C. Potter as partial fulfillment of the requirements for the degree of doctor of philosophy in food technology.
8. M. E. Wall *et al., J. Biol. Chem.* **198,** 533 (1952).
9. E. O. Ochiai, K. Tsuda, and S. Kitagawa, *Ber.* **70B,** 2083 (1937).
10. A. Meyer, O. Jaeger, and L. Ruzicka, *Helv. Chim. Acta.* **33,** 672 (1950).
11. We are indebted to Noyes Laboratory of Chemistry for the infrared analyses.
12. G. R. Lappin and L. C. Clark, *Anal. Chem.* **23,** 541 (1951).
13. G. C. Potter and F. A. Kummerow, in preparation.

8 March 1954.

Tumor Induction in *Drosophila melanogaster* by Injection of $e^{11}tu$ Larval Fluid

M. H. Harnly, L. Burton, F. Friedman, and M. J. Kopac

Department of Biology, Washington Square College New York University, New York

Hereditary melanotic tumors have been reported in *Drosophila melanogaster* by numerous workers (*1–9*). The expression of these pigmented growths is modified by nutrition (*2, 3, 7, 10, 11*), temperature (*12, 13*), irradiation with x-rays (*6, 14–16*), available oxygen (*15, 16*), estrogen-like compounds (*17*), and carcinogens (*18*).

A hereditary pigmented tumor arose spontaneously in our ebony stock of *Drosophila melanogaster*. This hereditary tumor is determined by a gene (*tu*) on chromosome two at approximately locus 88 (*19*). Depending upon the food available to the larvae or the temperature experienced by the larvae, the frequency of tumorous adults (homozygous) varies from 26 to 90 percent (*10, 12*). X-rays applied at the proper developmental stage also modify the penetrance of the tumor gene (*14*). Initial transplantation experiments of the pigmented growth into larvae of a nontumor strain indicated tremendous growth and subsequent appearance of multiple pigmented bodies. These findings were similar to those reported by Russell (*8*).

Both host and donor larvae of 120 hr of age (from egg laying) were chosen as test animals, because of the high postoperative survival of host animals at this age. Repeated trials were made for each test. For each trial, 50 donor larvae of the desired stock were used in the preparation of the larval extract. The larvae were washed 3 times in Waddington's insect Ringer's solution, placed in a mortar, and ground to an amorphous mash in 8 drops of insect Ringer's solution. The mixture of mashed larvae and insect Ringer's was pipetted into a centrifuge tube and centrifuged at 5400 rev/min for 15 min. The acellular supernatant fluid was then transferred into a clean, cooled tube and immediately placed in an ice bath. The acellular nature of the fluid was regularly confirmed microscopically by smears. The ice bath was employed to delay a blackening of the fluid extract that invariably occurred with the passage of time. The blackened fluid was so toxic that no animal injected with it ever survived the operation.

The 120-hr-old larval hosts were washed in three consecutive baths of insect Ringer's solution, dried, and etherized. The injection apparatus utilized in this experiment was essentially the same as that described by Ephrussi and Beadle (*20*), except that needle tips of very small diameter were used to reduce mortality. The needle was inserted at about the third or fourth segment from the posterior end of the larva. Fluid was administered until the larva was slightly distended. The host larvae were then placed on moist filter paper until they had fully recovered from the anesthetic. Only active larvae that possessed a scar

Table 1. Response to larval extract injections.

Donor	Host	Larvae tested	Dead larvae	Dead pupae			Adults tumorous
				No.	Tumorous	Emerged No.	
e tu	wild	160	66	40	39	54	54
*e tu**	wild	173	51	88	0	34	0
e tu	*y w*	52	7	22	22	23	19
wild	*y w*	38	9	10	0	19	0
y w	wild	67	18	12	0	37	0

* *e tu* donor reared on 2.5-percent "dead" brewer's yeast.

in the body wall that had been formed from the clot caused by the insertion of the needle were transferred to fresh vials of food. All other larvae were discarded. Thereafter all animals were observed daily.

Larvae that failed to pupate were removed and recorded without reference to the possible presence or absence of tumors, since they might have died before a possible tumor agent could have operated. Animals that died in the pupal stage were dissected, and the presence of tumors was recorded. All adults were dissected, and the presence and number of observable tumors per animal were recorded.

Three genotypes were used. The wild 51–52 stock had been inbred by single-pair sib matings for 51 generations and subsequently for 52 generations. It is a uniform stock, and not one tumorous imago was observed in more than 10,000 reared at 25°C on standard food, dissected, and examined (*21*). A yellow body, white eye (*y w*) stock maintained by mass matings in our laboratory was a random selection from our stocks and was used as a second test line. The ebony tumor strain was the third genotype used.

Insertion of the empty needle into the larva produced an operational injury that constituted a control test of the induction of tumors, and pure insect Ringer's solution was injected as a check on its activity as a tumor-inducing agent. Nearly 100 larvae of the tumor-free strains were tested by each of these methods, and none developed tumors.

The results of body fluid injections (Table 1) demonstrate that a melanotic-tumor-inducing agent was present in the 120-hr-old larvae of the ebony tumorous strain. Though somewhat diluted in extraction, it induced tumors in 93 of 94 genetically nontumorous wild-type animals and in 41 or 45 genetically nontumorous yellow white animals that survived long enough after the injection for tumors to develop (that is, through metamorphosis). The number of tumors formed in the wild-type adult hosts ranged from 1 to 24 tumors per animal, with a mean value of 3.7 melanotic tumors per adult fly. Conversely, the larval fluids of the wild 51–52 and yellow white nontumorous strains did not contain a tumor-inducing agent (Table 1).

Although these two strains neither possessed the tumor-inducing agent nor developed tumors under normal conditions, their larval tissue was reactive to the tumor-inducing agent of the ebony tumorous stock. This must mean that the tumor gene on chromosome two is synthesizing or controlling the synthesis of this tumor-inducing agent. It is specific to larval tissue, since it is incapable of inducing tumors in imaginal disk tissue. The degree of its specificity—that is, the types of larval tissue reactive to it—is being determined by histological studies (*22*). Whether this specificity actually resides in the agent or in the tissue is not yet known. If, in our other tumor strains, the hereditary tumors developing in imaginal disk (imago) tissue are due to an inducing agent, then it must be one of a different nature, since no larval tissue tumors are formed in these animals.

When first instar larvae of the ebony tumorous stock were raised on a sterile 2.5-percent "dead" brewer's yeast medium, 26 percent of the 580 emerged adults showed the pigmented tumor. This is in direct contrast to the fact that when similar aged larvae of the same stock developed on the normal cornmeal-molasses medium, 83 percent of the 726 emerged adults were tumorous. Therefore, extracts were made of the 120-hr-old larvae of the ebony tumorous strain that had been reared on sterile 2.5-percent "dead" brewer's yeast medium. These extracts were injected, in a series of three trials, into 120-hr-old larvae of the nontumorous 51–52 strain (Table 1). None of the 114 host animals surviving to metamorphosis possessed melanotic growths.

Injection of extracts obtained from donor larvae of the ebony tumorous strain that had been reared on a normal cornmeal-molasses medium produced tumors in 93 of the 94 injected wild 51–52 host larvae. When donor larvae were reared on the 2.5-percent "dead" brewer's yeast medium, extracts from these animals were not capable of inducing tumors in the wild 51–52 host animals. These facts indicate that the tumor-inducing capacity of donor larval extracts is modified by the nutrition available to developing larvae of the ebony tumorous strain.

References

1. C. B. Bridges, *Genetics* **1**, 107 (1916).
2. H. M. Briones, *Biologica* **10/11**, 55 (1949).
3. W. J. Burdette, *Texas Repts. Biol. and Med.* **3**, 123 (1950).
4. S. Ghelelovitch, *Compt. rend. acad. sci.* **230**, 1002 (1950).
5. M. H. Harnly, E. D. Goldsmith, and F. Friedman, *Cancer Research* **9**, 604 (1949) abst.
6. E. W. Hartung, *Cancer Research* **2**, 837 (1942).
7. I. H. Herskowitz and W. J. Burdette, *J. Exptl. Zool.* **117**, 499 (1951).
8. E. S. Russell, *J. Exptl. Zool.* **84**, 363 (1940).
9. M. B. Stark, *J. Cancer Research* **3**, 279 (1919).
10. F. Friedman, M. H. Harnly, and E. D. Goldsmith, *Cancer Research* **11**, 904 (1951).
11. S. Ghelelovitch, *Compt. rend. acad. sci.* **232**, 1776 (1951).
12. G. C. Emery and M. H. Harnly, master's thesis, N.Y.U. (1951).
13. E. W. Hartung, *J. Exptl. Zool.* **106**, 223 (1947).
14. M. L. Fleischer and M. H. Harnly, master's thesis, N.Y.U. 21 (1954).
15. B. Glass and H. L. Plaine, *Proc. Natl. Acad. Sci. U.S.* **38**, 697 (1952).
16. H. L. Plaine and B. Glass, *Cancer Research* **12**, 829 (1952).
17. D. Brncic and S. Koref, *Science* **114**, 492 (1951).

18. W. J. Burdette, *Cancer Research* **12**, 366 (1952).
19. M. H. Harnly *et al.*, *Cancer Research* **11**, 254 (1951) abst.
20. B. Ephrussi and G. W. Beadle, *Am. Naturalist* **70**, 218 (1936).
21. L. Burton and M. H. Harnly, master's thesis, N.Y.U. (1953).
22. M. L. Kaplan and M. H. Harnly, master's thesis, N.Y.U. (1954).

13 April 1954.

Serologic Differences in Strains of Herpes Simplex Virus

C. Ray Womack and Brewster P. Hunt

Department of Microbiology, Louisiana State University School of Medicine, New Orleans

Previous serologic studies have yielded conflicting evidence on the occurrence of antigenic differences among strains of herpes simplex virus. In some cross-neutralization tests in mice (*1*) and in mouse and chick embryos (*2*), antigenic differences were considered to have been demonstrated. No serologic differences were noted, however, in the strains examined in other observations involving neutralization in chick embryos (*3, 4*) and in infant mice (*5*) or employing cross-complement fixation (*4, 6*). In none of these experiments did the number of strains tested exceed four.

Current opinion appears to favor the belief that strains of herpes virus are remarkably uniform in their serologic behavior. Nevertheless, puzzling immunologic behavior has been observed occasionally (*7, 8*), suggesting that antigenic dissimilarity of herpes strains may indeed exist.

Table 1. Cross-complement fixation titers with six strains of herpes simplex and their antiserums.

Antiserum vs.	Antigens					
	AS	AH	O'C	HF	RH	WG
AS	8*	4	8	0	0	0
AH	16	16	16	4	0	8
O'C	16	16	64	8	8	8
HF	8	8	8	8	0	4
RH	4	8	8	4	32	32
WG	0	0	4	0	8	32

* Reciprocal of serum titer.

In order to examine the latter possibility, immune serums were obtained from rabbits that survived corneal infection with 11 strains of herpes virus that had been adapted to growth in the chick embryo. Nine of these were adapted to the chick embryo following isolation in eggs and two (HF and O'C) were laboratory strains previously passed in rabbits or mice. All showed the usual biologic properties of herpes simplex virus, and all but three produced symptomatic encephalitis in the rabbit.

Cross-complement fixation tests have been carried out between six of these viruses and their antiserums. All antiserums were tested simultaneously against a single antigen. Antigens consisted of bacteriologically sterile amniotic fluids from chick embryos dying after yolk-sac inoculation. Their optimal dilution was determined by a grid titration (antigen dilutions versus serum dilutions) with the homologous antiserum. Two minimal hemolytic units of complement were used in the tests, which were completed after 18-hr over-night fixation at 4°C by the addition of sensitized cells. The end-point chosen was 3+ fixation (50 percent hemolysis). The usual controls for specificity of the reactions were carried out simultaneously with the tests.

The titers obtained in the cross serologic examination of the six strains are presented in Table 1. A calculation of 2-titer ratios (*9–11*) yields the values appearing in Table 2. A ratio of 50-percent similarity in strains (ratio ½) or greater indicates relative serologic identity of strains, and a relationship of less than 50 percent (ratio $< \frac{1}{2}$) is required for demonstration of serologic differences. It is evident that, even in this limited group of strains, striking antigenic differences obtain between the closely related strains AS and AH on the one hand and the closely related strains RH and WG on the other. The other two strains are intermediate in their relationships to these extremes. Recent studies by Jawetz *et al.* (*12*) support the view that antigenic dissimilarity in herpes strains has been clearly demonstrated.

Additional observations on the relationship of these strains to others are being carried out by cross-complement fixation tests, and cross-neutralization tests along similar lines are in progress.

References and Notes

1. H. B. Slavin and E. Gavette, *Proc. Soc. Exptl. Biol. Med.* **63**, 345 (1946).
2. A. L. Florman and F. W. Trader, *J. Immunol.* **55**, 263 (1947).
3. F. M. Burnet and D. Lush, *J. Pathol. Bacteriol.* **48**, 275 (1939).

Table 2. Cross antigenic relationships of six strains of herpes simplex. First figures refer to 2-titer antigenic ratios. Values in parentheses are percentages of relationship between strains.

	AS	AH	O'C	HF	RH	WG
AS	1 (100)					
AH	$\frac{1}{1.4}$ (71.4)	1 (100)				
O'C	$\frac{1}{2}$ (50.0)	$\frac{1}{2}$ (50.0)	1 (100)			
HF	$\frac{1}{2.8}$ (35.7)	$\frac{1}{2}$ (50.0)	$\frac{1}{2.8}$ (35.7)	1 (100)		
RH	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{5.7}$ (17.5)	$\frac{1}{8}$ (12.5)	1 (100)	
WG	$\frac{1}{16}$ (6.3)	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{8}$ (12.5)	$\frac{1}{2}$ (50.0)	1 (100)