few exceptions among young mice which died, apparently of anemia, with hemoglobin levels of less than 4 g/100 ml, at under 50 days of age. Otherwise, both individually and collectively,

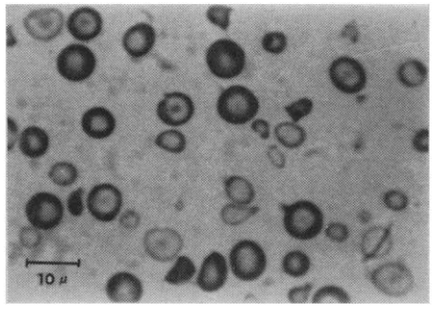

Fig. 1. Blood smear of a young, affected, male *sla* mouse, showing variable red cell morphology, with anisocytosis, poikilocytosis, polychromasia, cell fragments, and target cell formation.

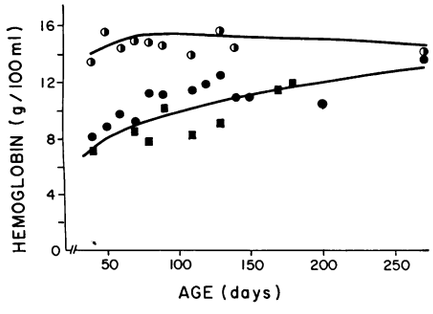

Fig. 2. Hemoglobin concentrations in hemizygous *sla* males (■), homozygous *sla* females (●), and heterozygous *sla* female carriers (◑), at different ages. Each point is the mean value from groups of 6 to 12 animals observed serially.

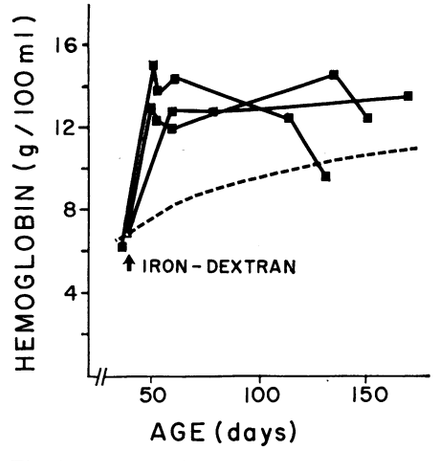

Fig. 3. Hemoglobin concentrations in three anemic *sla* mice after single intraperitoneal injections of iron-dextran (0.01 ml; 1 mg Fe; diluted with saline immediately before injection), compared with the expected curve from Fig. 2 (broken line).

hemoglobin levels rise towards normal values (Fig. 2), and the morphologic picture tends to become more nearly normal. These findings are in contrast to Grewal's original observations (*2*). The anemia occurs and tends to correct itself with time when the mice are on a standard laboratory diet containing apparently adequate amounts of iron, pyridoxine, and other substances (see *3*).

Heterozygous female carriers do not show any significant anemia (Fig. 2), but minor morphologic changes in blood smears can often be detected.

The striking morphologic appearances in the anemic mice prompted us to try treatments with various substances. Anemic *sla* mice were treated with intraperitoneal injections of androgens [which may stimulate release of endogenous erythropoietin (*4*)], pyridoxine [since human pyridoxine-responsive anemia is characterized by a similar morphologic picture (*5*)], iron-dextran, dextran alone, and saline alone. The only treatment to produce an effect was iron-dextran, after which there was an unmistakable and sustained rise in hemoglobin levels toward normal values (Fig. 3).

Preliminary experiments with Fe^{59} indicate that the absorption of inorganic iron is in the same range as in normal, nonanemic mice. The utilization of injected tracer doses of Fe^{59} for hemoglobin formation is also within the normal range in preliminary studies. Determinations of total body iron show that it is not reduced in anemic mice. The transferrin pattern observed by starch-gel electrophoresis is the same as that of normal mice, as reported previously (*6*), and we have been able to demonstrate by autoradiography and by direct counting that it binds Fe^{59}.

Despite a tendency to correction with time, and apparent amelioration by intraperitoneal administration of relatively massive amounts of iron as iron-dextran, this anemia appears not to be due to a simple deficiency of iron nor to defective iron absorption. It is likely that the mechanism may be a more unusual primary defect in iron metabolism.

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References and Notes

1. D. S. Falconer and J. H. Isaacson, *Genet. Res. Camb.* **3**, 248 (1962).
2. M. S. Grewal, *ibid.*, p. 238.
3. Rockland mouse diet reported to contain Fe, 0.019 percent, and pyridoxine, 209 to 275 μg/100 g.
4. C. W. Gurney and W. Fried, *J. Lab. Clin. Med.* **65**, 775 (1965).
5. D. L. Horrigan and J. W. Harris, in *Advances in Internal Medicine*, W. Dock and I. Snapper, Eds. (Year Book Medical Publishers, New York, 1964), vol. 12, pp. 103–174.
6. B. L. Cohen, quoted by Grewal (*2*).
7. Supported by PHS grant AM 05581-03S1 and grant G-63-BGH-8-1 from the United Health Foundation of Western New York. We are indebted to D. S. Falconer for the breeding stock and for advice and encouragement.

1 November 1965

Simian Virus 40: Replication in the Presence of Specific Antiserum and Adenovirus 4

Abstract. *Twelve consecutive passages of simian virus 40, made in the presence of adenovirus 4 and antiserum to simian virus 40, indicated that simian virus 40 would multiply almost indefinitely under these conditions. The adenovirus and the simian virus had previously replicated together in the absence of antiserum. These data support the conclusion that the SV-40 genome is incorporated within the protein coats of adenovirus 4.*

We have reported the bizarre phenotypic properties of simian virus 40 (SV-40) which had originally contaminated a strain of adenovirus 4 and which subsequently persisted after six cell-culture passages in the presence of antiserum to SV-40. This contaminant, SV-40, possessed the antigenic and thermal stability properties of the adeno-

Table 1. Titers of SV-40 and adenovirus 4 during seven consecutive passages in tissue culture. The titers of SV-40 are given as $\log_{10}$ $TCID_{50}$ per 0.2 ml of culture supernatant, titrated in cercopithecus kidney cell monolayers. The titers of adenovirus 4 are given as $\log_{10}$ $TCID_{50}$ per 0.2 ml culture supernatant, titrated in human-embryo kidney cell monolayers.

Passage No.	Cell type	Titer		Cumulative dilution ($\log_{10}$)	Passage duration (days)
		SV-40	Adenovirus 4		
6	CMK*	3.1		0	
7	HEK†	3.0		−1.3	5
8	HEK	4.0	5.5	−2.6	3
9	HEK	3.3	5.8	−4.3	2
10	HEK	3.5	4.5	−6.0	2
11	HEK	2.5	5.0	−7.7	3
12	CMK	3.0	5.5	−9.4	5

* Cercopithecus kidney cell monolayers. † Human-embryo kidney cell monolayers.

virus and not of SV-40; it was concluded that the SV-40 genome was included within the protein coats of adenovirus. We now report on further passages of the contaminant SV-40 in the presence of adenovirus 4 and antiserum to SV-40.

Details concerning antiserum, virus, and methods of viral assay have been described (*1*). The antiserum had a titer of 1:20,000 against 100 $TCID_{50}$ (tissue-culture infective doses, 50 percent effective) of SV-40, and was present in a concentration of 1:250 in passages No. 7 through No. 11. No additional antiserum was added to passage No. 12, although in this passage antiserum was carried over from passage No. 11. In addition to titration in cell cultures of *Cercopithecus aethiops* kidney cultures, the various passages were titrated in human-embryo kidney cells to assess the true concentration of the adenovirus (*2*). The 6th and the 12th SV-40 passages were in cercopithecus kidney cells, while the intervening passages were in human-embryo kidney cells. Frozen, thawed, and centrifuged virus suspension was added to SV-40 antiserum (diluted 1 : 5), and the mixture was incubated at 36°C for 0.5 hour prior to inoculation into the next succeeding culture. As a control, $10^{5.5}$ $TCID_{50}$ of SV-40, obtained by end-dilution of passage No. 6 in cercopithecus cells and free of any large amounts of adenovirus 4, were treated in a manner analogous to that in passages No. 7 through No. 12; that is, all passages were made in the presence of SV-40 antiserum.

Table 1 shows that the titers of SV-40 and adenovirus 4 remained relatively constant in the seven passages shown. The cumulative dilution of the sample from passage No. 6, by the time it reached passage No. 12, was over a billion fold. Thus, not only did SV-40 resist the action of antiserum to SV-40 after it had replicated in the presence of adenovirus 4, but it also multiplied at a seemingly constant rate. In contrast, the SV-40 from passage No. 6, freed of adenovirus 4, had a titer of only $10^{0.3}$ $TCID_{50}$ per 5 ml in passage No. 7; none could be detected in subsequent passages. This showed that the SV-40 alone was rapidly neutralized by its homologous antiserum.

The titers of SV-40 and adenovirus 4 in the various passages bear a fixed ratio to one another, an indication that the velocity constants for the replication of both viruses are similar. Incorporation within coat proteins of adenovirus 4 may not only confer on SV-40 the phenotypic properties described (*1*), but may also enable small amounts of SV-40 to replicate in human embryo-kidney cell cultures with a facility approximating that of the adenovirus itself.

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References and Notes

1. J. M. Easton and C. W. Hiatt, *Proc. Nat. Acad. Sci. U.S.* **54**, 1100 (1965).
2. G. T. O'Conor, A. S. Rabson, I. K. Berezesky, F. J. Paul, *J. Nat. Cancer Inst.* **31**, 903 (1963); A. S. Rabson, G. T. O'Conor, I. K. Berezesky, F. J. Paul, *Proc. Soc. Exp. Biol. Med.* **116**, 187 (1964).
3. We thank Dr. J. L. Bittle, Lederle Laboratories, for provision of virus stocks and antiserum and C. A. Robinson for technical assistance.
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Major Components in the Exocrine Secretion of a Male Butterfly (Lycorea)

Abstract. *Extracts of the extrusible secretion-disseminating organs ("hairpencils") of the male of the danaid butterfly,* Lycorea ceres ceres, *from Trinidad, contain a pyrrolizidine and two aliphatic esters. An odorous component, present in trace amounts, remains unidentified. Judging from the function of "hairpencils" in a related species, the secretion may play a mediating role in courtship.*

Courtship, mating, and other social interactions of animals are frequently mediated by substances called pheromones, secreted by the animals themselves (*1*). Among the very few pheromones that have been isolated and identified are the sex attractants of the silkworm moth (I) and gypsy moth (II). No studies have been made on butterflies, despite the fact that these insects are often endowed, in one sex or the other, with exocrine glands that might logically be presumed to secrete pheromones (*2*). The males of the subfamily Danainae (family Nymphalidae) possess a pair of extrusible brushlike structures, the "hairpencils," which because of their noticeable odor have long been suspected to act as scent-disseminating organs, and which are, in fact, associated with secretory cells at their base. In the queen butterfly, *Danaus gilippus berenice*, whose courtship has recently been investigated in detail (*3*), the hairpencils are protruded by the males during aerial pursuit of the female and are brushed against her head and antennae. In response to this behavior, the female alights on available herbage, while the male hovers above her and continues to "hairpencil" her

$$CH_3(CH_2)_2CH{=}CHCH{=}CH(CH_2)_8CH_2OH$$

(I)

$$CH_3(CH_2)_5\underset{\displaystyle OCCH_3\\ \displaystyle \|\\ \displaystyle O}{CH}CH_2CH{=}CH(CH_2)_5CH_2OH$$

(II)

anterior end. Eventually he settles beside her and copulation occurs.

As a first step in a comparative study of the hairpencil secretion of a variety of danaid butterflies, we are reporting on the chemistry of the secretion of one of them, *Lycorea ceres ceres*, from Trinidad. The hairpencils of this species are particularly large (Fig. 1) and richly endowed with secretion.

The tufts of hairs from hairpencils of about 300 live *Lycorea* males (*4*) were pulled off with forceps and extracted with methylene chloride or carbon disulfide. The infrared spectrum of this extract showed strong absorption at 3.45, 3.52, 5.77, 5.92, and 8.10 μ, and gas-liquid chromatography (GLC), on a column of 5 percent SE-30 silicone gum at 180°C, showed three major components (retention times 1.7, 10.5, and 20 minutes). Fractional sublimation gave a crystalline compound with the characteristic sweetish odor of the natural secretion. This component, mp 74° to 75°C, had infrared bands at 3.4, 5.95, and 6.45 μ. In the ultraviolet, a maximum at 288 mμ (log ϵ 4.22, based on the molecular weight of 135 determined by mass spectrometry) implied the presence of a conjugated system. These data speak for the molecular formula C_8H_9ON. A nuclear magnetic resonance spectrum at 100 Mcy/sec clearly confirmed the presence of nine protons, disposed as follows: a three-proton singlet at τ 7.80, a pair of two-proton triplets (the coupling constant $J \cong 6.5$ cy/sec at τ 7.16 and 5.87, and a pair of less well-resolved one-proton doublets ($J \cong 2.5$ cy/sec) at τ 3.91 and 3.31.