100 mg% glucose. The rate of increase was greater in medium with 100 mg% glucose than in 50 mg% glucose.

Trypsinization of confluent fibroblasts followed by the addition of 100 mg% glucose medium, without diluting the cell number per dish, did not change the cellular glycogen concentration (data not shown). The decrease in glycogen concentration during the first day after subcultivation was further investigated by refeeding confluent fibroblasts with media containing various concentrations of glucose in lieu of subcultivation (figure 3). 50% of the stored glycogen disappeared from the cells refed with HTU-MEM within 2 h and 97% within 8 h. From 1 to 20 mg% glucose, the rate of glycogen depletion was a function of the concentration of medium glucose. In 50 mg% glucose, the glycogen content did not change. The glycogen content of cells refed with 100 mg% glucose remained unchanged for the first 8 h and then increased 30% during the next 16 h.

Discussion. Glycogen storage and metabolism by human fibroblasts have been studied in glycogen storage diseases[2,3] and in normal controls[4]. The glycogen content for cultures in 50 and 100 mg% glucose increased as the cells approached confluency (figure 2). Seiter and Summer[3] and DiMauro et al.[2] also demonstrated that the glycogen content increased starting 3–5 days after subcultivation, but they did not provide data for early logarithmic growth. The present data showed that when confluent fibroblasts were dispersed and diluted in fresh medium with 100 mg% glucose the glycogen content per mg protein decreased during the early logarithmic phase of growth (figure 2). The rate of depletion of stored glycogen was a function of the glucose concentration (figures 2,3) although the cells continued to grow at a normal rate for 5 days in all media (figure 1). No decrease in glycogen concentration was observed when confluent cells were refed with 100 mg% glucose (figure 3). The trypsinization procedure itself had no effect on the glycogen content. Therefore, the decrease in glycogen observed after subcultivation was a reflection of the growth rate of the cells.

The glycogen content of cells grown in medium with low glucose, or in HTU-MEM which lacked glucose, was depleted within 8–24 h after refeeding. This indicates that glycogen has a very limited potential as an energy source. Under conditions in which the medium glucose and cellular glycogen are depleted, the cells utilize noncarbohydrate energy sources such as glutamine[6,9]. Therefore, the availability of glycogen is apparently not a requirement for cell growth or for maintaining cell viability but rather is a characteristic of in-vivo cells retained by fibroblasts.

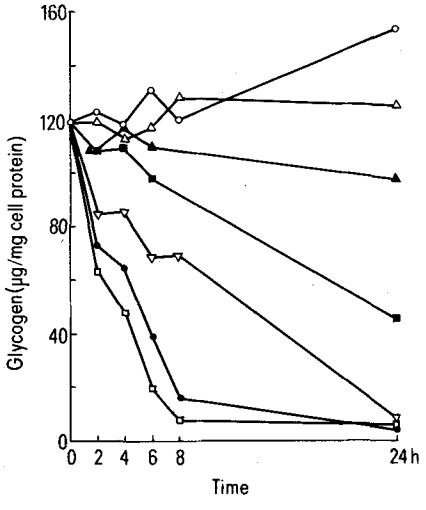

Fig. 3. Glycogen levels after refeeding of confluent cells grown in 100 mg% glucose with media containing different concentration of glucose. 100 mg% glucose (○), 50 mg% glucose (△), 20 mg% glucose (▲), 10 mg% glucose (■), 5 mg% glucose (▽), 1 mg% glucose (●) and HTU-MEM (□).

1 Supported in part by a grant from the Frank G. Bressler Fund.
2 S. DiMauro and W.J. Mellman, Pediat. Res. *7*, 745 (1973).
3 C.W. Seiter and G.K. Summer, Proc. Soc. exp. Biol. Med. *149*, 945 (1975).
4 V.J. Cristofalo, B.V. Howard and D. Kritchevsky, in: Organic Biological and Medicinal Chemistry, vol. II, p. 95. Ed. U. Gallo and L. Santamaria. Noord Hollandsche Uitgevers MIJ, Amsterdam 1970.
5 V.J. Cristofalo and D. Kritchevsky, J. cell. Physiol. *67*, 125 (1966).
6 H.R. Zielke, P.T. Ozand, J.T. Tildon, D.A. Sevdalian and M. Cornblath, Proc. nat. Acad. Sci. USA *73*, 4110 (1976).
7 W.D. Lust, J.V. Passonneau and S.K. Crites, Analyt. Biochem. *68*, 328 (1975).
8 H.O. Lowry, J.N. Rosebrough, L.A. Farr and J.R. Randall, J. biol. Chem. *193*, 265 (1951).
9 H.R. Zielke, P.T. Ozand, J.T. Tildon, D.A. Sevdalian and M. Cornblath, J. cell. Physiol. *95*, 41 (1978).

Cardiac glycosides in *Danaus chrysippus* (L.) provide some protection against an insect parasitoid

D.A.S. Smith

Department of Biology, Eton College, Windsor, Berkshire SL4 6EW (England), 23 December 1977

Summary. The larvae of *Danaus chrysippus* are less susceptible to attack by endoparasitic Diptera of the family Tachinidae if they feed on plants containing cardiac glycosides.

The Old World queen butterfly, *Danaus chrysippus* (L.) (Danaidae), is subject to severe attack by endoparasitic flies (Diptera) of the family Tachinidae[1,2]. In Sierra Leone nearly 100% of larvae were found to be infected at the start of the dry season[1] although in Ghana the peak frequency was only 8% and other parasites predominated[2].

In mid-April 1975 a wild population of *D. chrysippus* at Dar es Salaam, Tanzania, which had been under study since early 1972, suffered a major plague of tachinids which had scarcely abated by August when breeding was terminated. High mortality occurred even in broods reared indoors which was exceptional for in the previous 3 years only one

tachinid had turned up in the laboratory. As the butterfly eggs were obtained in muslin sleeves and transferred thence to insect-proof cages prior to hatching, the infection must have resulted from fly eggs deposited on the food-plant outdoors and subsequently ingested when fed to the caged caterpillars. The tachinid larva emerges from the butterfly pupal case, after biting an escape hole, to form its puparium elsewhere. Thus, both pupal mortality and fly numbers are easily scored.

38 broods of larvae from single females were reared from April to August 1975 (table) and fed on 1 of 5 different food-plants. Mortality was very significantly higher on *Tylophora stenoloba*, which contains no cardiac glycosides[3], than on *Calotropis gigantea* which is a rich source[4] ($d = 11.4$; $p < 0.001$). 2 broods were split equally at hatching between *Tylophora* and *C. gigantea*, the main alternative food-plants of the local wild population: pupal mortality on the former was 100% ($n = 23$) and on the latter 54.2% ($n = 24$) ($X_1^2 = 11.3$; $p < 0.001$). The data for the 3 introduced toxic food-plants, which were grown intermingled in the same flowerbed, are less straightforward. On both *C. procera* and *Gomphocarpus*, which are rich in cardenolides[4], mortality was low. The very high mortality on *Asclepias curassavica*, normally a toxic species[4], might imply that this Kilimanjaro strain, which has not been tested, is poor in cardenolides. This interpretation is supported by the small size of the 6 surviving butterflies ($\bar{x}$ for forewing length = 37.0 mm), scarcely larger than the *Tylophora* specimens ($\bar{x} = 35.4$ mm)[5] and very significantly smaller ($t_{34} = 4.7$; $p < 0.001$) than others raised on the Munich strain of *A. curassavica* ($\bar{x} = 41.1$ mm)[5] which is known to be toxic[4].

Proportions of *D. chrysippus* pupae parasitized by tachinid flies while feeding as larvae on various Asclepiadaceae

Food-plants	No. of pupae	No. parasitized	Percent parasitized
Calotropis gigantea[a,d]	191	46	24.1
Calotropis procera[b,e]	45	15	33.3
Asclepias curassavica[b,f]	87	81	93.1
Gomphocarpus fruticosus[b,g]	29	2	6.9
Tylophora stenoloba[c,d]	160	136	85.0
Totals	511	277	54.2

[a] This strain contains cardenolides[4]. [b] This species contains cardenolides[4] but strain not tested. [c] Cardenolides absent[3]. [d] Plants naturally established at Dar es Salaam. [e] Seeds collected from Same, Pare District, Tanzania. [f] Seeds collected from Marangu, Kilimanjaro District, Tanzania. [g] Seeds obtained from Auckland, New Zealand by Dr W.B. Rudman.

The association between mortality and food-plant is due either to differences in the number of eggs deposited on the leaves by the flies or to the caterpillars being protected in varying degrees according to the quantity of cardenolide sequestered from the food-plant. Whichever cause is the true one, and they are not mutually exclusive, wild butterflies laying upon *C. gigantea* and other poisonous species, with the exception of *Asclepias* which grows very poorly in Dar es Salaam, must have a strong selective advantage over those using *Tylophora* during a tachinid outbreak. My results show that enhanced protection against parasites may be an important selective attribute to be won by danaids which feed on toxic plants. This would be emphatically so if, as has been suggested[2], parasites are more important than predators in regulating the butterfly population.

1 D.F. Owen, Tropical butterflies. Clarendon Press, Oxford 1971.
2 M. Edmunds, Zool. J. Linn. Soc. *58*, 129 (1976).
3 E. Abisch and T. Reichstein, Helv. chim. Acta *45*, 2090 (1962).
4 M. Rothschild, J. von Euw, T. Reichstein, D.A.S. Smith and J. Pierre, Proc. R. Soc. *B 190* (1975).
5 D.A.S. Smith, Experientia *34*, 845 (1978).

The effect of cardiac glycoside storage on growth rate and adult size in the butterfly *Danaus chrysippus* (L.)

D.A.S. Smith[1]

Department of Biology, Eton College, Windsor, Berkshire SL4 6EW (England), 23 December 1977

Summary. Danaus chrysippus grows faster and attains significantly greater size when fed as a larva on several species of Asclepiadaceae (milkweeds) containing cardiac glycosides than on one which does not.

Danaus chrysippus (L.) (Danaidae) is an abundant African, Asian and Australasian tropical butterfly[2] which is aposematic, frequently distasteful[3] and a model for numerous mimics, both Batesian and Müllerian, from at least 5 families of Lepidoptera[3,4]. Most of its numerous food-plants belong to the Asclepiadaceae (milkweeds) and contain heart poisons (cardiac glycosides or cardenolides), substances which have toxic (cardioactive), noxious and emetic properties[3,5]. Cardenolides are sequestered by the larvae and often stored in the pupae, adults and eggs[3,6], all of which may thereby acquire protection from predators which not only find them distasteful but also often experience emesis[7,8]. I now report that larvae of *D. chrysippus* fed upon toxic species of milkweed produce an adult of significantly greater body size compared with those reared on a nontoxic asclepiad, *Tylophora stenoloba*. Similar results have been reported for *D. plexippus*[9].

Eggs were obtained from *D. chrysippus* at Dar es Salaam, Tanzania, by sleeving females outdoors on the appropriate food-plant. Subsequently, the larvae were reared indoors on the same species collected either from the natural habitat (*Calotropis gigantea* and *T. stenoloba*) or from a cultivated plot (*Calotropis procera, Asclepias curassavica* and *Gomphocarpus fruticosus*).

My results (table), based on forewing length, which correlates well with body mass in both sexes, show that males are larger than females by 0.8 mm ($t_a = 6.6$; $p < 0.001$). Variance analyses indicate that the size differences between butterflies reared on the various plant species are highly significant for both males ($F = 34.8$, d.f. $^3/_{428}$; $p < 0.001$) and females ($F = 21.2$, d.f. $^4/_{547}$; $p < 0.001$). The only individually significant comparisons in t-tests (all giving $p < 0.001$) are for butterflies fed on each of the toxic species[3] on the one hand and on *Tylophora*, which contains no cardenolides[10], on the other. Comparing means of the combined toxic plant samples with the *Tylophora* sample, the size advantage to the former is 5.9 mm ($t_a = 15.5$; $p < 0.001$) for males