# Experimental Section

Gerontology *26:* 241–246 (1980)

# Effect of Treatment with Phenformin, Diphenylhydantoin or *L*-Dopa on Life Span and Tumour Incidence in C3H/Sn Mice

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**Key Words.** Mice · Phenformin · Diphenylhydantoin · *L*-Dopa · Life span · Tumour incidence

**Abstract.** The chronic treatment of female C3H/Sn mice with phenformin (2 mg/day) and diphenylhydantoin (2 mg/day) prolonged mean life span by 23 and 25%, respectively, and decreased spontaneous tumour incidence by 4.0 and 2.3 times, respectively. The chronic treatment of mice with *L*-dopa (2 mg/day) did not change these parameters and decreased the multiplicity of mammary tumours. The mechanisms of the drug action on mouse life span and tumour incidence are discussed.

At present, a large-scale search for means to prolong the life span of higher organisms, man included, is underway. It is generally believed that natural ageing does not constitute a key factor in the formation of specific age pathology and, therefore, it should not be expected that life span prolongation and inhibition of ageing-associated processes may be effected by taking the same measures (*Hayflick*, 1976). However, there is a considerable body of evidence pointing to a correlation between the process of natural ageing and specific age pathology (*Dilman*, 1971, 1979). This approach suggests administration of preparations, capable of (a) lowering the threshold of sensitivity of hypothalamopituitary complex to regulatory stimuli, and (b) reducing the level of utilization of free fatty acids (FFA) for energy supply, to influence both processes. Since phenformin is especially capable of reducing FFA utilization for energy supply, it was proposed for administration with a view to slow down the rate of atherosclerosis and cancer development (*Dilman*, 1971, 1979). The antiepileptic drug, diphenylhydantoin, which inhibits the secretion of insulin and glucocorticoid hormones, may be instrumental in dealing with ageing-associated metabolic disturbances (*Dilman et al.*, 1975). Finally, these two drugs as well as *L*-dopa reduce the threshold of sensitivity of the hypothalamopituitary complex to inhibition by estrogens (*Dilman and Anisimov*, 1975), i.e.,

**Table I.** Life span and spontaneous tumour incidence in female C3H/Sn mice treated with phenformin, diphenylhydantoin or *L*-dopa during their lifetime

| Drug | Number of animals | Life span | | Number of tumour bearing mice | | Number of tumours |
|---|---|---|---|---|---|---|
| | | mean | maximal | total | % | |
| Control | 30 | 450 ± 19 | 641 | 24 | 80.0 | 41 |
| Phenformin | 25 | 555 ± 32[2] | 810 | 5 | 20.0[2] | 5 |
| Diphenylhydantoin | 23 | 558 ± 28[2] | 782 | 8 | 34.8[2] | 9 |
| *L*-Dopa | 22 | 493 ± 39 | 810 | 14 | 63.6 | 17 |

[1] 4 polyps of endometrium, 1 thecafolliculoma of ovary, adenomyoma of uteri.

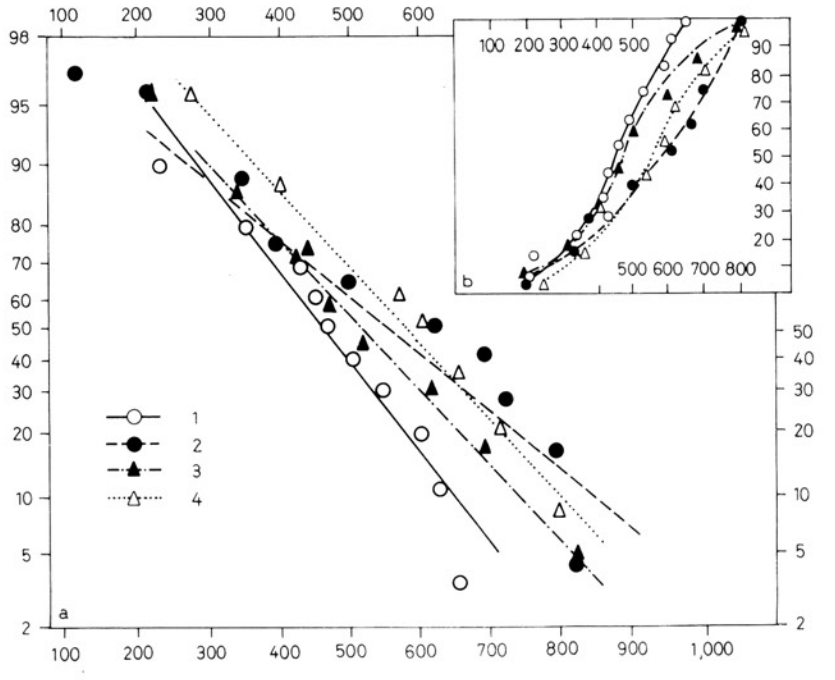


**Fig. 1.** Effect of phenformin, diphenylhydantoin or *L*-dopa treatment on life span in female C3H/Sn mice. **a** Linear anamorphoses of survival rate curves (probit-scale). **b** Mortality curves. y axis = %; x axis = age, days; 1 = control; 2 = phenformin; 3 = *L*-dopa; 4 = diphenylhydantoin. Symbols indicate every third animal.

| Mean tumour latency period days | Adenocarcinomas of mammary gland | | | | Leukemia number of animals | | Other tumours, number of tumour-bearing mice |
|---|---|---|---|---|---|---|---|
| | number of animals | | number of tumours | | | | |
| | total | % | total | per mouse | total | % | |
| 471 ± 22 | 19 | 63.3 | 30 | 1.58 | 5 | 18.7 | 6[1] |
| 499 ± 122 | 4 | 16.0[2] | 4 | 1.00 | 1 | 4.0 | – |
| 549 ± 46 | 7 | 30.4[2] | 7 | 1.00 | 2 | 8.7 | – |
| 493 ± 39 | 11 | 50.0 | 13 | 1.18 | 3 | 13.6 | 1[3] |

[2] Difference as compared with control is significant, $p < 0.05$. [3] Polyp of endometrium.

they influence one of the factors responsible for the development of homeostatic disturbances with increasing age (*Dilman*, 1971, 1979).

## Method

100 female C3H/Sn mice, aged 3.5 months, were supplied from the Rappolovo Animal Farm of the USSR Academy of Medical Sciences. The animals were kept in plastic cages, 7–8 animals in each cage, in natural light and received standard chow and tap water *ad libitum*. All mice were divided into four groups, and received either 0.2 ml of tap water *per os* (control) or 2 mg of phenformin (Phenethylbiguanide, Dibotin, England), 2 mg of diphenylhydantoin (Diphenin, officinal drug, USSR) or 2 mg of *L*-dopa (3,4-dioxyphenylalanin, Levodopa, Yugoslavia) per mouse, in the same volume of water, five times a week, during the whole survival period. The animals were examined by palpation weekly to detect mammary gland tumours. Autopsies were carried out on all dead animals and those sacrificed in a poor condition. Neoplastic tissues were examined microscopically. Experimental results were given a statistical treatment using the Student's t test and $\chi^2$. Survival rate and tumour yield curves were converted to linear anamorphoses with the aid of probit analysis procedures (*Storm*, 1970).

## Results

Life-long administration of phenformin and diphenylhydantoin was shown to result in an appreciable increase (by 23 and 25%, respectively, $p < 0.05$) in the life span of the mice (table I). The linear anamorphoses of the survival rate curves (fig. 1) show that the effect of diphenylhydantoin is not associated with changes in the rate of ageing. However, the decrease of the slope of the linear anamorphose of the survival rate curve for phenformin-treated mice points to a delayed ageing process. The treatment with these two preparations was followed by a significant decrease in tumour incidence rates (4.0 and 2.3 times, respectively, $p < 0.05$). Administration of these drugs resulted in a reduced frequency and multiplicity of mammary adenocarcinomas (table I; fig. 2). The *L*-dopa dose employed in this study did not produce any appreciable effect on life span duration or frequency of mammary neoplasms. Its effect was limited to a reduced multiplicity of these tumours (1.18 vs. 1.58 in control). Apart from phenformin, all the drugs had no effect on the frequency of leukaemia.

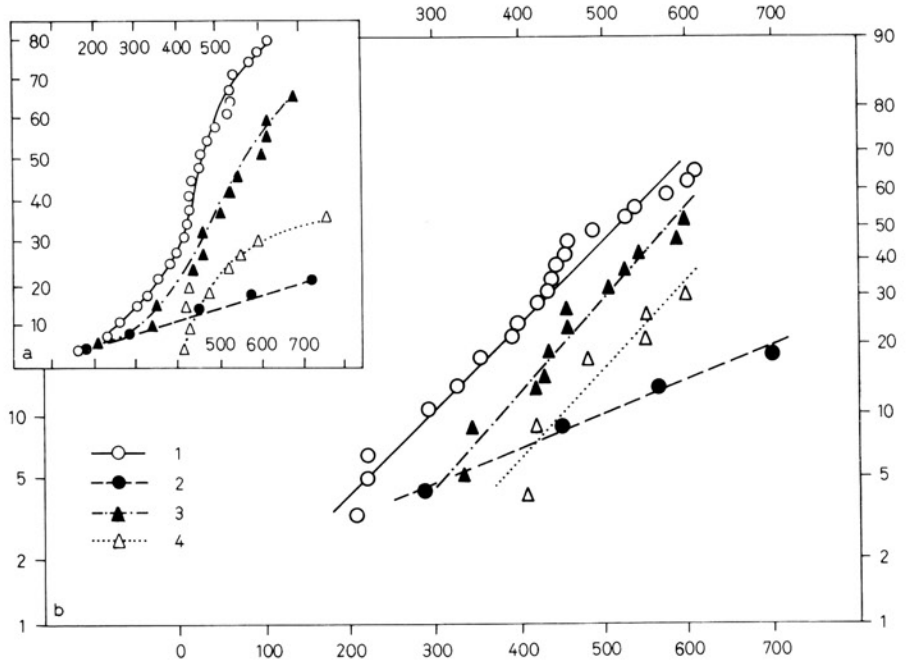


**Fig. 2.** Effect of phenformin, diphenylhydantoin or *L*-dopa treatment on tumour development in female C3H/Sn mice. **a** Dynamics of all tumour development. **b** Linear anamorphoses of curves of mammary tumour yield. y axis = % (fig. b, probit scale); x axis = age, days; 1 = control; 2 = phenformin; 3 = *L*-dopa; 4 = diphenylhydantoin. Symbols indicate each tumour-bearing animal.

## Discussion

Hence, treatment with diphenylhydantoin and, to a greater extent, with phenformin produces a significant reduction in tumour incidence in experimental mice and prolongs their life span, whereas administration of 2 mg of *L*-dopa does not exert such effect. These findings indicate that carcinogenesis induced by viruses and, particularly, that of MTV is subject to modification by different factors of a systemic character. Such modifying effect on mammary carcinogenesis is generally attributed to changes in hormonal balance, particularly, the decreased production of sex hormones or prolactin (*Welsch and Nagasawa,* 1977). However, since phenformin treatment not only cut down the incidence of mammary tumours but also all other types of neoplasms, leukaemia included, it may be supposed that in the final analysis the anti-tumour effect of phenformin, which is actually a lipostatic drug (*Muntoni,* 1974), is similar to that of restricted-calorie and low-fat diets (*Carrol and Khor,* 1975; *Fernandes et al.,* 1976). This suggestion is further supported by both the reduction in tumour incidence and prolongation of the animals' life span. It should be taken into account that phenformin-induced normalizing of lipid-carbohydrate metabolism leads to the annulment of metabolic immunodepression both in man (*Dilman et al.,* 1977a) and rats in which immunity was inhibited by carcinogen substances (*Dilman et al.,* 1977b).

The ability of diphenylhydantoin to lower insulin and glucocorticoid secretion (*Dilman et al.*, 1975) may be an important factor of its action. It is noteworthy in this respect that an anahormone of ACTH, capable of inhibiting glucocorticoid production, can reduce mammary tumour incidence in C3H mice (*Bulovskaya and Krylova*, 1975). Finally, it cannot be ruled out that the reducing effect of phenformin and diphenylhydantoin treatment on the threshold of sensitivity of hypothalamopituitary complex to inhibition by estrogens (*Dilman and Anisimov*, 1975) is actually related to the rise in the concentration of biogenic amines in the brain, especially that of dopamine. Dopamine is known to inhibit the secretion of prolactin, one of the major modifiers of mammary carcinogenesis in rodents (*Welsch and Nagasawa*, 1977). However, in our experiments, the administration of *L*-dopa, an immediate precursor of dopamine and, therefore, an inhibitor of prolactin secretion, failed to affect the incidence of mammary tumours, although their multiplicity diminished and the linear anamorphose of the tumour-yield curve shifted to the right (fig. 2). The ineffectiveness of *L*-dopa treatment is perhaps due to the dosage used in our experiments (about 60–90 mg/kg), because other authors reported an effective inhibition of DMBA-induced mammary carcinogenesis by *L*-dopa administration to rats (*Welsch and Nagasawa*, 1977). It should be mentioned that prolongation of mouse life span was attained by treatment with much higher doses of *L*-dopa (5,000 mg/kg), which, however, caused toxic side effects (*Cotzias et al.*, 1974).

Hence, to summarize, treatment with phenformin and diphenylhydantoin may both prolong life span and reduce tumour incidence in C3H/Sn mice. This furnishes another argument in support of the correlation between ageing and diseases of ageing, e.g., cancer. It should be pointed out that these two processes were inhibited simultaneously with the aid of a calorie-restricted diet (*Fernandes et al.*, 1976), antioxidants (*Emanuel and Obukhova*, 1978) and, finally, by treatment with phenformin and diphenylhydantoin. Since all these measures have an effect on the different stages of accumulation or utilization of FFA in the body, it seems desirable to study the interplay of their effects.

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Received: July 31, 1979
Accepted: August 30, 1979

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