Table 1. Body weight, red cell count, nerve conduction velocities and EEG data in control and lead intoxicated, male and female 35-week-old rats

			Controls		Tests	
			Males	Females	Males	Females
Body weight (g)			582±71 (9)	312±17 (8)	524±44 (9)	303±24 (7)
Red cell count ($\times 10^{12}$/l)			7.4±0.3 (5)	5.8±1.1 (5)	7.7±0.5 (5)	6.4±0.3 (5)
Sciatic nerve conduction velocity (motor) (m/sec)			55±13 (5)	55±8 (5)	51±9 (5)	57±14 (5)
Sciatic nerve conduction velocity (sensory) (m/sec)			70±4 (5)	62±2 (5)	65±4 (5)	62±1 (5)
EEG	Arousal	NW	8-10 Hz (diffuse), β (frontal) (3)		8-10 Hz (diffuse), β (frontal) (3)	
		AW	7 Hz (parieto-occipital), β (frontal) (3)		7 Hz (parieto-occipital), β (frontal) (3)	
	Sleep	SW	2-5 Hz (diffuse) (3)		2-5 Hz (diffuse) (3)	
		SP	14 Hz, high voltage (diffuse) (3)		14 Hz, high voltage (diffuse) (3)	
		PS	7-8 Hz (diffuse) (3)		7-8 Hz (diffuse) (3)	

Values are mean±SD. The number of animals is indicated in parentheses. Differences between control and test group are in no case statistically significant ($p > 0.05$). Abbreviations: NW, normal waking; AM, active waking; SW, slow waves; SP, spindles; PS, paradoxical sleep.

Table 2. Plasma, red cells, brain areas and sciatic nerve lead levels in 8-month-old rats intoxicated by lead acetate (0.2% in drinking water) since birth

	Control rats		Test rats	
	a	b	a	b
Plasma	<0.05	<0.2	0.12±0.04	0.6±0.2
Red cells	<0.05	<0.2	2.26±0.26	10.9±1.2
Pons medulla	<0.1	<0.5	0.50±0.17	2.4±0.8
Cerebellum	<0.1	<0.5	0.56±0.06	2.7±0.3
Midbrain	<0.1	<0.5	0.50±0.07	2.4±0.3
Hypothalamus	<0.1	<0.5	0.48±0.19	2.3±0.9
Striatum	<0.1	<0.5	0.56±0.08	2.7±0.4
Hippocampus	<0.1	<0.5	1.00±0.27	4.8±1.3
Cerebral cortex	<0.1	<0.5	1.42±0.30	6.8±1.4
Sciatic nerve	<0.1	<0.5	0.22±0.13	1.1±0.6

a) Lead levels in µg/ml or µg/g fresh weight; b) lead levels in µM or nmoles/g fresh weight. Values are mean±SD. n=4 animals for each value.

lead content is higher in the less mature areas and that lead progressively accumulates in hippocampus and neocortex as the animal matures and the intoxication endures.

The reason for lead accumulation in those areas is not obvious. It has been reported that lead concentrates in capillaries[17-19] and it is known that the surface of endothelium is larger in cerebral neocortex than in other regions of the nervous system[20]. Therefore, the high lead content in cerebral cortex may in part be a consequence of a larger endothelial cell compartment with a high lead content. If lead has such a compartmentation, the total content in a given area will give little indication about the lead level to which brain cells are actually exposed.

2 Michaelson, I.A., Toxic. appl. Pharmac. *26* (1973) 539.
3 Press, M.F., J. Neuropath. exp. Neurol. *36* (1977) 169.
4 Holtzmann, D., Herman, M.M., Shen Hsu, J., and Mortell, P., Virchows Arch., Path. Anat. *387* (1980) 147.
5 Lefauconnier, J.M., Hauw, J.J., and Bernard, G., Neuropath. exp. Neurol. *42* (1983) 177.
6 Hrdina, P.D., Hanin, I., and Dubas, T.C., in: Lead Toxicity, p.273. Eds R.L. Singhal and J.A. Thomas. Urban and Schwartzenberg, Wien 1980.
7 Campbell, J.B., Woolley, D.E., Vijayan, V.K., and Overmann, S.R., Dev. Brain Res. *3* (1982) 595.
8 Grandjean, P., Toxic. Letters *2* (1978) 65.
9 Scheuhammer, A.M., and Cherian, M.G., Neurotoxicology *3* (1982) 85.
10 Frederickson, C.J., Manton, W.I., Frederickson, M.H., Howell, G.A., and Mallory, M.A., Brain Res. *246* (1982) 338.
11 Fjerdingstad, E.J., Danscher, G., and Fjerdingstad, E., Brain Res. *80* (1974) 350.
12 Danscher, G., Hall, E., Fredens, K., Fjerdingstad, E., and Fjerdingstad, E.J., Brain Res. *94* (1975) 167.
13 Collins, M.F., Hrdina, P.D., Whittle, E., and Singhal, R.L., Toxic. appl. Pharmac. *65* (1982) 314.
14 Stowe, H.D., Goyer, R.A., Krigman, M.D, Wilson, M., and Cates, M., Arch. Path. *95* (1973) 106.
15 Okazaki, H., Aronson, S.M., Di Maio, D.J., and Olvera, J.E., Trans. Am. neurol. Ass. *88* (1963) 248.
16 Klein, M., Namer, R., Harpur, E., and Corbin, R., New Engl. J. Med. *283* (1970) 669.
17 Toews, A.D., Kolber, A., Hayward, J., Krigman, M.R., and Morell, P., Brain Res. *147* (1978) 131.
18 Thomas, J.A., Dallenbach, F.D., and Thomas, M., J. Path. *109* (1973) 45.
19 Stumpf, W.E., Sar, M., and Grand, L.D., Neurotoxicology *1* (1980) 593.
20 Bradbury, M., in: The Concept of a Blood-Brain Barrier, p. 20. John Wiley, Chichester-New York 1979.

1 Services d'explorations fonctionnelles du système nerveux, Hôpital Saint Antoine, F-75012 Paris, France.

0014-4754/83/091030-02$1.50+0.20/0

Regeneration and in vitro flowering of plants derived from callus cultures of opium poppy *(Papaver somniferum)*[1]

T. Yoshikawa and T. Furuya[2]

School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108 (Japan), September 6, 1982

Summary. From callus cultures of *Papaver somniferum* L., green buds and shoots were formed at a high rate under illumination at low temperatures, 16-18 °C. The shoots continued to grow and finally flowered in vitro and in soil.

The opium poppy, *Papaver somniferum* L., is an important medicinal plant, which contains morphinan alkaloids, such as morphine, codeine and thebaine. Various calluses have successfully been induced from the capsule and the other parts of the plant. However, it was shown from the chemical constituents[3] and by biotransformation experiments[4]

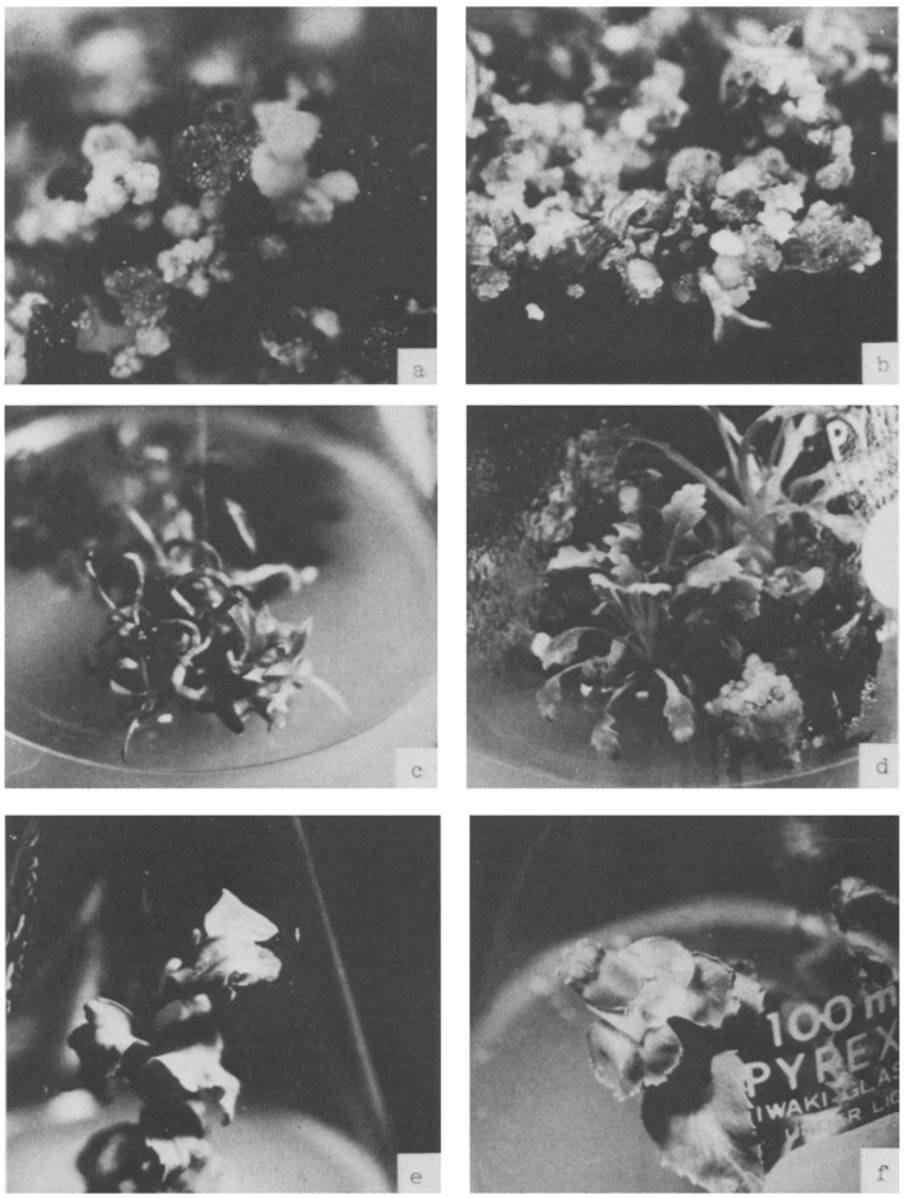

Figure 1. In vitro regeneration of plantlets from callus cultures of *Papaver somniferum*. *a* Albino callus spontaneously appeared in the original callus tissues. *b* Green buds and shoots developed from albino callus under illumination at 16 °C. *c* Plantlets developed from green buds (b) at 26 °C, which had subulate and entire leaves. *d* Clonal plantlets developed from shoots (b) at 16 °C, which had serrate and lobate leaves. *e* In vitro flowering of plantlets regenerated from callus tissues, which had 4 curly petals, a pistil and many anthers. *f* Fully matured capsule on 2nd week after flowering.

that the calluses were deficient in the ability to biosynthesize morphinan alkaloids. Recently, 2 groups of investigators have reported the production of morphinan alkaloids in the callus cultures of *P. somniferum*; one identified only codeine[5] and another both thebaine and codeine[6]. However, it was suggested that these calluses partially differentiated a tracheary element under illumination and/or in the presence of high concentrations of cytokinin. In fact, Tam et al.[5] describe the existence of tracheids in the callus and Kamo et al.[6] report the restoration of the ability to biosynthesize morphine in the shoot regenerated from the callus.

We have tried to elucidate the relationship between the potential to biosynthesize secondary metabolites and the appearance of structural features, i.e. differentiation. With some papaveraceae such as *P. somniferum* it has proved especially difficult to regenerate the mature plant from the callus[7]. In this experiment, however, we successfully obtained the plantlets and flowers in vitro and in soil from the callus tissues of *P. somniferum*.

In the experiments described the callus tissues were induced from the hypocotyls of 10-day-old seedlings of *Papaver somniferum* L. var. Ikkanshu on Murashige and Skoog's medium containing with 2,4-D (2,4-dichlorophenoxyacetic acid) 1 ppm, kinetin 0.1 ppm and 7% coconut milk. Albino callus as shown in figure 1a appeared in high frequency in the original callus following transfer to the medium without coconut milk. The albino callus, which was first separated in our laboratory[7] and after a few years named meristemoids by Nessler et al.[8], had smaller cells, more dense cytoplasm, higher content of lipid and higher potential for differentiation than the original one. Subsequently, the callus was subcultured on the same medium as above at 3-week intervals for a year and transferred to MS medium supplemented with 0.1-1.0 ppm kinetin under illumination of about 4000 lx for 16 h, and 8 h darkness, at 16–18 °C. After 1 generation, green buds and shoots (fig. 1,b) were formed at a high frequency at low temperatures, 16–18 °C. The shoot formation was actively promoted at the low temperatures, 16–18 °C, but inhibited at higher temperatures, above 25 °C. The shoots which occasionally developed at the high temperature did not grow more than 1.5–2.0 cm in height, and the leaves were abnormal and had a subulate shape (fig. 1,c) On the other hand, the shoots developed at the low temperature grew to 10–15 cm in

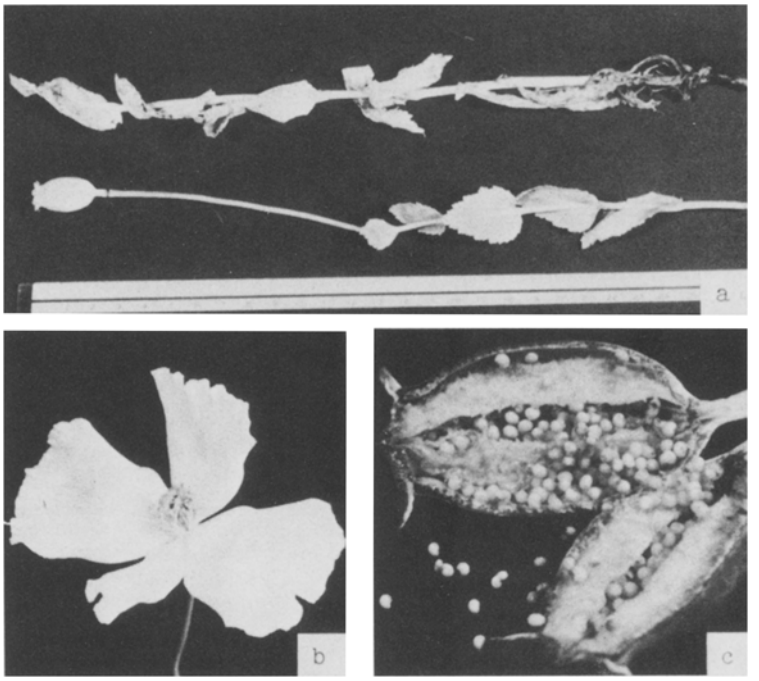

Figure 2. Plantlets developed in soil after regeneration from callus cultures of *Papaver somniferum*. *a* The plantlets cultivated for 3 months after transferring into soil in pot from clonal shoots (fig. 1,d). *b* The flower of a plantlet developed in soil from clonal shoots, which had 4 different sizes of petals. *c* The mature capsule of a plantlet developed in soil, which contained many seeds.

height and the leaves were normal and a serrate shape (fig. 1,d). Finally, the regenerated plants flowered in vitro under the optimal light conditions, 16 h photoperiod at about 7000 lx. The flower formed in vitro from the callus continued to blossom for 3 weeks (fig. 1,e), although that of the original plant grown from a seedling fell within a day. The flower generated in vitro had 4 white petals, a pistil and many anthers, but the petals were curly and the capsule, which developed fully from the pistil (fig. 1,f), did not contain any seed and latex. This fact suggests that the pollen was not formed in the anthers or remained immature.

On the other hand, the plantlets which formed roots in vitro (in the step of fig. 1,d) were transferred into soil in pots, and grew to about 50 cm in height after 3 months (fig. 2,a). The flower then produced closely resembled those of the original plant, except that one pair of petals was extremely small as compared to the other one (fig. 2,b). The fully matured capsule was about 2 cm in height and 1.5 cm in width and contained the latex and many seeds (fig. 2,c). Recently, we confirmed that the seeds had the potential to germinate. Moreover, we found that various tissues which regenerated from the callus cultures contained the morphinan alkaloids. The main alkaloid found was codeine, although the original plants produced morphine as the major alkaloid[9]. The morphinan alkaloid content of each tissue in various differentiation steps is under investigation.

1 Part 41 in the series 'Studies on plant tissue cultues'. For Part 40 see Furuya, T., Yoshikawa, T., Orihara, Y., and Oda, H., J. natl Prod. (1983) in press.
2 To whom reprint requests should be addressed.
3 Furuya, T., Ikuta, A., and Syōno, K., Phytochemistry *11* (1972) 3041.
4 Furuya, T., Nakano, M., and Yoshikawa, T., Phytochemistry *17* (1978) 891.
5 Tam, W.H.J., Constabel, F., and Kurz, W.G.W., Phytochemistry *19* (1980) 486.
6 Kamo, K.K., Kimoto, W., Hsu, A-F., Mahlberg, P.G., and Bills, D.D., Phytochemistry *21* (1982) 219.
7 Ikuta, A., Syōno, K., and Furuya, T., Phytochemistry *13* (1974) 2175.
8 Nessler, C.L., and Mahlberg, P.G., Can. J. Bot. *57* (1979) 675.
9 Yoshikawa, T., and Furuya, T., in: Plant tissue culture 1982, p. 307. Ed. A. Fujiwara. Maruzen, Tokyo 1982.

0014-4754/83/091031-03$1.50 + 0.20/0

Effect of alloimmunized thymic cells on isolated mouse atria. Participation of prostaglandins[1]

A.M. Genaro, G.A. Cremaschi, M.E. Sales and E.S. Borda[2]

Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Serrano 665/69, 1414 Buenos Aires (Argentina), October 25, 1982

Summary. The effects of thymic cells from alloimmunized mice on the mechanical activity of isolated mouse atria were explored. Immune cells decreased the tension without changing the rate of beating of the atrium. After inhibition of prostaglandin synthesis with indomethacin and acetylsalicylic acid, the negative inotropic action of alloimmunized thymic cells was blocked.

The heart has been shown to release in vitro a number of oxidative metabolites of arachidonic acid (AA) in response to changes in oxygen tension[3], hormone stimulation[4], nerve stimulation[5,6], and mechanical damage[7], and during cardiac anaphylaxis[8].

The synthesis of prostaglandins (PGs) and related lipids is