Effect of preoperative stress on serum cholesterol in surgical patients

Age group and number of subjects	Serum cholesterol in mg (%) On admission (O. A.)	Pre-operative (Pre. op.)	Post-operative (p. o.)	On discharge (O. D.)
A 11–20 years (n = 7)	152.8 ± 12.7	177.14 ± 13.57	148.85 ± 7.39	123.71 ± 8.25
B 21–30 years (n = 30)	151.33 ± 7.35	178.60 ± 8.96	155.10 ± 7.93	127.66 ± 6.42
C 31–40 years (n = 14)	159.21 ± 13.22	182.93 ± 15.91	153.93 ± 12.51	130.93 ± 9.98
D 41–50 years (n = 9)	157.22 ± 12.21	192.11 ± 12.87	163.77 ± 12.96	122.44 ± 12.27
E 51–60 years (n = 5)	140.8 ± 20.2	176.2 ± 17.75	134.6 ± 15.96	122.8 ± 21.4

Statistical analysis (Paired t-t test)
Mean values ± SE.

Age gr.	O.D. vs. O.A.	O.D. vs. Pre.op.	O.A. vs. Pre.op.	Pre.op. vs. P.O.	O.D. vs. P.O.
A	t=3.86 p<0.01**	t=5.257 p<0.01**	t=3.37 p<0.05*	t=2.44 p<0.05*	t=3.73 p<0.01**
B	t=5.49 p<0.001***	t=7.54 p<0.001***	t=12.44 p<0.001***	t=6.23 p<0.001***	t=6.69 p<0.001***
C	t = 3.83 p<0.01**	t = 5.68 p<0.001***	t=4.8 p<0.001***	t=4.93 p<0.001***	t=5.35 p<0.001***
D	t=4.05 p<0.01**	t=8.30 p<0.001***	t=5.2 p<0.001***	t=4.81 p<0.01**	t=4.9 p<0.01**
E	t=1.67 p<1 NS	t=4.98 p<0.01**	t=3.24 p<0.05*	t=4.07 p<0.05*	t=2.92 p<0.05*

NS = not significant; *significant; **very significant; ***highly significant.

of serum cholesterol level was 43.5, 39.9, 39.0, 56.9 and 43.4 in age groups A, B, C, D, E respectively. Examination of results also indicates rise of serum cholesterol level on the day of admission as compared with that on the day of discharge in all age groups except group E. Post-operatively there is a consistant fall in serum cholesterol level although it is still significantly higher than that on the day of discharge. Cholesterol level at the time of discharge from hospital served as control value.

Discussion. Examination of serum cholesterol level at the time of discharge for all age groups in this series reveals low values. Observed low serum cholesterol levels can be attributed to the fact that majority of patients attending Civil Hospital for treatment purposes belonged to low socioeconomic group. Patients were found to be mainly vegetarian and subsisting on low dietary cholesterol intake. Elevated serum cholesterol levels on the day of admission as compared with that on the day of discharge indicate the traumatic effect of hospitalization. Statistically insignificant rise of cholesterol in group E on the day of admission as compared with that on the day of discharge revealed by paired t-test, suggests better mental preparedness. However, preoperatively there is a statistically significant rise of serum cholesterol as compared with that on the day of admission.

This clearly shows that preoperative anxiety and fear remain the same in all age groups. The plasma cholesterol normally varying between 150 and 250 mg per cent is known to be influenced by factors like diet, age, hormones, genetic predisposition, emotional conditions and physical activities[15]. Investigations of fluctuations of cholesterol level depending upon the time of the day, have also been reported[16,17]. Patients of this series were operated within 1 or 2 days of admission. Hospital diet on which they subsisted was vegetarian and of low fat-cholesterol content. Dietary cholesterol takes several days to equilibrate with cholesterol in plasma and several weeks to equilibrate with tissue cholesterol[18]. Determination of cholesterol level in serum of the patients after recovery was carried out by collecting blood samples at same hour as collected on the operation day. This revealed fluctuation of the order of 2 to 3% which confirms that the observed rise of cholesterol in this series is definitely due to preoperative stress only. Rise of serum cholesterol level in preoperative stress can be due to increased sympathetic activity-catecholamine secretion resulting in increased 'VLDL' secretion by liver, involving extra triglyceride and cholesterol output into circulation[18]. In this series there was no patient with renal impairment, the condition which is known to result in elevated plasma cholesterol level. Present investigation clearly indicates the significance of preoperative plasma cholesterol rise as a simple useful guide in assessment of stress.

15 T. G. Minlin and K. Chen, Drug Res. *1*, 128 (1959).
16 S. H. Rubin, J. biol. Chem. *131*, 619 (1939).
17 H. Buhl, Münch. Med. Wschr. *108*, 2490 (1960).
18 H. A. Harper and P. Mayes, in: Review of Physiological Chemistry, 14th ed., p. 268 (1973).

Toxicity of *Phytophthora infestans* and *Alternaria solani* to chick embryos[1]

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Summary. None of the extracts and culture filtrates from growth of 9 races of *Phytophthora infestans* on living potato tissue and potato dextrose broth are toxic to chick embryos. Chloroform extracts of 4 out of 10 isolates of *Alternaria solani* grown on potato dextrose broth showed some toxicity to chick embryos.

Under ideal conditions potato tubers are perfectly harmless and fit for human consumption, however, they do contain traces of toxic compounds. Some of the identified toxic compounds include glycoalkaloids. Birth defects such as anencephaly and spina bifida have been attributed to unidentified substances in blighted potatoes[2]. However, rats fed blighted potato material have failed to show such malformations[3–6]. Testing of blighted,

Table 1. Toxicity of extracts and culture filtrates of *Phytophthora infestans* grown on potato dextrose broth (PDB) and surface sterilized potato slices

Race	Medium	Extract	Hatchability (%)	Extract	Hatchability (%)
Control	PDB	–	95 ns	Chloroform	90 ns
(uninoculated)	Potato	Water	95 ns	Chloroform	100 ns
0	PDB	Culture filtrate	90 ns	Chloroform	100 ns
	Potato	Water	95 ns	Chloroform	95 ns
4	PDB	Culture filtrate	100 ns	Chloroform	100 ns
	Potato	Water	100 ns	Chloroform	90 ns
1.2.3	PDB	Culture filtrate	95 ns	Chloroform	90 ns
	Potato	Water	90 ns	Chloroform	100 ns
1.2.4	PDB	Culture filtrate	85 ns	Chloroform	100 ns
	Potato	Water	100 ns	Chloroform	95 ns
1.3.4	PDB	Culture filtrate	100 ns	Chloroform	90 ns
	Potato	Water	95 ns	Chloroform	95 ns
2.3.4	PDB	Culture filtrate	100 ns	Chloroform	95 ns
	Potato	Water	90 ns	Chloroform	95 ns
1.2.3.4.	PDB	Culture filtrate	100 ns	Chloroform	100 ns
	Potato	Water	100 ns	Chloroform	90 ns
1.2.3.4.6.7.8	PDB	Culture filtrate	95 ns	Chloroform	95 ns
	Potato	Water	95 ns	Chloroform	90 ns
1.2.3.4.7.8	PDB	Culture filtrate	90 ns	Chloroform	95 ns
	Potato	Water	100 ns	Chloroform	100 ns

ns, Not significantly different from control at 5% level.

Table 2. Toxicity of extracts and culture filtrates of *Alternaria solani* grown on potato dextrose broth (PDB) and surface sterilized potato slices

Isolate	Medium	Extract	Hatchability (%)	Extract	Hatchability (%)
Control	PDB	–	100 ns	Chloroform	90 ns
(uninoculated)	Potato	Water	95 ns	Chloroform	100 ns
A-1	PDB	Culture filtrate	90 ns	Chloroform	0*
	PDB			Chloroform ($^1/_{10}$ conc)	20*
	Potato	Water	90 ns	Chloroform	95 ns
C-1	PDB	Culture filtrate	100 ns	Chloroform	85 ns
	Potato	Water	90 ns	Chloroform	90 ns
C-3	PDB	Culture filtrate	90 ns	Chloroform	30*
	Potato	Water	85 ns	Chloroform	90 ns
C-4	PDB	Culture filtrate	100 ns	Chloroform	95 ns
	Potato	Water	100 ns	Chloroform	100 ns
C-5	PDB	Culture filtrate	95 ns	Chloroform	90 ns
	Potato	Water	90 ns	Chloroform	100 ns
C-6	PDB	Culture filtrate	100 ns	Chloroform	90 ns
	Potato	Water	95 ns	Chloroform	90 ns
Mi-6	PDB	Culture filtrate	85 ns	Chloroform	15*
	Potato	Water	100 ns	Chloroform	100 ns
Ma-9	PDB	Culture filtrate	90 ns	Chloroform	40*
	Potato	Water	90 ns	Chloroform	100 ns
Ma-10	PDB	Culture filtrate	100 ns	Chloroform	85 ns
	Potato	Water	100 ns	Chloroform	90 ns

ns, Not significantly different from control at 5% level. * Significantly different from control at 1% level.

aged, and control potatoes failed to produce spina bifida or anencephaly in rats, rabbits, hamsters or mice[7]. This study was conducted to investigate the toxicity to chick embryos of several races and isolates of *Phytophthora infestans* and *Alternaria solani* which caused late and early potato blight.

Materials and methods. 9 races of *Phytophthora infestans* and 10 isolates of *Alternaria solani* collected from several researchers in Europe and USA were used for toxicity study. 9 races of *Phytophthora infestans* included race 0, 4, 1.2.3, 1.2.4, 1.3.4, 2.3.4, 1.2.3.4, 1.2.3.4.6.7.8 and 1.2.3.4.7.8. 10 isolates of *Alternaria solani* included A-1, C-1, C-3, C-4, C-5, C-6, Mi-6, Ma-9 and Ma-10. All the cultures were maintained on freshly prepared potato dextrose agar at 20 °C and transferred to new media every 2 weeks.

2 kinds of media, autoclaved potato dextrose broth and surface sterilized potato slices, were used to grow fungi for toxicity study. Mycelia of each culture from agar slant were transferred to 100 ml potato dextrose broth in 500-ml Erlenmeyer flasks and incubated at 20 °C for 3 weeks. 10 flasks were used for each culture to provide sufficient material for extraction. For preparation of blight potatoes, Russet Burbank tubers were first washed by soaking in 0.1% sodium hypochlorite for 1 h. Tubers were then peeled and cut into slices (about 1 cm × 1 cm × 5 cm). Surface sterilization was achieved by soaking potato slices in 2% sodium hypochlorite for 3 min and then rinsing them 3 times with sterilized water. Surface sterilized potato slices were then put into quarter size sterilized Mason jars and capped with 3 layers of sterilized Whatman No. 1 filter paper. Each jar held about 500 g potato slices. For inoculation mycelia collected from 2-week-old culture in culturing flasks were disrupted by slow blending of mycelia with sterilized water. Inoculation was accomplished by pouring 30 ml mycelial mixture into each jar. Rotating the jars made uniform contacting of potato slices with mycelia. Inoculated potato was then incubated at 20 °C for 2 weeks and then freeze-dehydrated by using a pilot scale freeze dryer.

After incubation, 10 ml of culture filtrate from potato broth was removed. The remaining material was mixed and extracted with chloroform with the aid of a high speed mixer. The extraction procedure was repeated three times, using 150 ml chloroform for each extraction. The chloroform extracts were combined, concentrated in a flask evaporator, and diluted to 5 ml with chloroform. For blighted potatoes, 500 g freeze dehydrated slices were extracted with chloroform and water. The chloroform extracts were combined and concentrated to 5 ml. The water extracts were also combined but concentrated to 10 ml.

Chicken egg air sac inoculations[8] were used to assay the toxicity of culture filtrates, chloroform and water extracts. Groups of 10 fertile White Leghorn eggs were

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2 J. H. Renwick, Br. J. Prev. Soc. Med. *26*, 67 (1972).
3 D. E. Poswillo, D. Sopher and S. Mitchell, Nature *239*, 462 (1972).
4 S. Chaube, C. A. Swinyard and R. H. Daines, Lancet *1*, 329 (1973).
5 R. F. Keeler, D. R. Douglas and G. F. Stallknecht, Proc. Soc. exp. Biol. Med. *146*, 284 (1974).
6 J. A. Ruddick, J. Harwig and P. M. Scott, Teratology *9*, 165 (1974).
7 R. F. Keeler, D. R. Douglas and G. F. Stallknecht, Am. Potato J. *52*, 125 (1975).
8 M. J. Verrett, J. P. Marliac and J. McLaughlin, J. Ass. off. agric. Chem. *47*, 1003 (1964).

inoculated each with 0.02 ml of the test materials before incubation. Duplicate groups were tested for each sample material. Control groups were inoculated with sterilized potato dextrose broth, chloroform extract of potato dextrose broth, or water and chloroform extract of uninoculated potato slices. Analysis of variance was made and means were compared according to Tukey's ω-procedure[9]. Honest significant difference (HSD) was used to judge the significance between control and treated.

Results and discussion. Table 1 shows that none of the extracts and culture filtrates from growth of 9 races of *Phytophthora infestans* on living potato tissue and potato dextrose broth had any toxicity to check embryos. This was shown by the nonsignificant difference in hatchability between control and extract treated fertile eggs. Four out of the 10 isolates of *Alternaria solani* showed some toxicity to chick embryos (table 2). These included isolate A-1, C-3, Mi-6 and Ma-9. Among these, chloroform extract of A-1 grown on potato dextrose broth was the most toxic. It is interesting to know that only chloroform extracts from *Alternaria solani* isolates grown on potato dextrose broth showed some toxicity. Culture filtrates of potato dextrose broth and chloroform and water extracts of *Alternaria solani* infected living potato tissue did not have toxicity to chick embryos. Surface sterilized potato tissue supported luxury growth of *Alternaria solani*. Apparently, some inhibitor(s) in the living potato tissue inhibited the formation of toxin(s) by *Alternaria solani* and the inhibitor(s) must have been heat-labile. Also, the toxin(s) must have been water insoluble since only chloroform extracts showed toxicity. Chicken egg air sac inoculations[8] were generally used for screening of mycotoxins produced by fungi. The results together with the finding by other researchers[3-7] suggest that consumption of blighted potaoes is unlikely to create a hazard as far as public health is concerned.

9 R. G. D. Steel and J. H. Torrie, Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, N.Y. 1960.

An escape phenomenon from water and sodium retention induced by propranolol in rats

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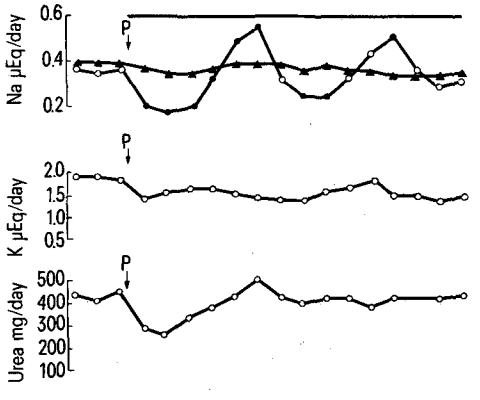
Summary. Rats given a daily dose of propranolol 45 mg/kg b.wt retain water and sodium for 4 days, escaping during the 5th and 6th days in which their excretions are larger than basal values. Afterwards, in the period studied, they make a new retention and clearing is less accentuated. No relationship could be found between these retentions and plasma renin activity or renal renin content.

Propranolol (Sumial®, ICI-Farma, Pontevedra), a known β-blocker, has been proved to be able to lower the secretion of renin from the juxtaglomerular apparatus[2,3]. Also an effect of propranolol (P) in reducing diuresis and Na and urea excretion has been recently observed[4].

Female Wistar rats (weight 242 ± 16 g, average ± SEM) were placed into individual metabolic cages and allowed free access to standard rat food and tap water. During 4 days (basal period), all rats received an i.m. injection of 0.25 ml glucose 5% twice a day. From the 5th day (day 0 of experiment) water was substituted by 20 ml (average of water intake on basal days) of a solution containing P 9 mg/kg b.wt and a i.m. injection of P 18 mg/kg b.wt was given twice a day. Every day water and food consumption were recorded and clean urine collected under mineral oil. Urinary volume was measured and Na and K contents analyzed by flame photometry (IL 143, Instrumentation Laboratory, Boston, Mass. USA). Urinary urea was measured with an AutoAnalizer (Technicon).

A group of 10 animals was studied during 19 days (4 basal + 15 P) in order to control water and salt balance. A second group of 25 animals identically treated were sacrified by lots of 5 animals previously to the P administration the days 4 and 10 (maximal retention) and 7 and 13 (maximal excretion). They were lightly anesthetized with sodium pentobarbital (Nembutal, Abbot) and after renal pedicle ligation, blood samples were taken by aortic puncture for Plasma Renin Activity (PRA[5]) and creatinine[6] measurements. Kidneys were removed, weighted and Renal Renin Content (RRC) was determined[7].



Na, K and urea excretion on each day of the experiment. Propranolol (P) is administered from the 4th day (arrows) to the end of the experiment. Each point is the average of 10 animals. ▲, Intake; ○, excretions; ●, excretions significantly different from the intake (in Na excretion figure only).

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2 F. R. Bühler, J. H. Laragh, E. D. Baer, Jr, and H. R. Brunner, New Engl. J. Med. *287*, 1209 (1972).
3 A. M. Michelakis, R. G. McAllister, J. clin. Endocr. Metab. *34*, 386 (1972).
4 F. J. Ortega, J. M. López-Novoa, M. A. Rengel and L. Hernando, submitted to Eur. J. Clin. Invest.
5 E. Haber, T. Koesner, L. Page, B. Kliman and A. Purnode, J. clin. Endocr. *29*, 134 (1969).
6 P. D. Doolan, E. L. Alpen and G. B. Theil, Am. J. Med. *32*, 65 (1972).
7 J. Menard and K. J. Catt, Endocrinology *90*, 422 (1972).