From Tables I and II it would be seen that range of variation in alkaloid content was maximum in Coondapur collections from 1·526% to 2·034% and was lowest in graft seedlings which were older in age. Age, however, could not be a handicap since Dutta *et al.*[3] did not observe significant differences in alkaloid content in roots of varying ages indicating that higher age does not result appreciably, either in increase or decrease in its total alkaloid. Alkaloid content in Coondapur material was significantly higher at 1% level than the Mangalore stocks while, at 5% level, than the graft seedlings. Within Coondapur itself (Table III) there were location-wise variations in alkaloid content. Plants from two places, *viz.*, Seethanady and Thingale were rich in alkaloid, containing more than 2%, which was nearing that in the control.

TABLE II

T-test significance for different R. serpentina *stocks*

Stocks	Haldwani stock	Haliyal	Mangalore	Coondapur
Graft seedling	0·12	0·87	0·68	2·65*
Haldwani stock	..	0·27	0·00	1·52
Haliyal	..	..	0·81	1·30
Mangalore	..	.	..	3·12**

* Significant at 5%; ** Significant at 1%.

TABLE III

Mean alkaloid content in plants from different localities in Coondapur

Locality	Alkaloid %	Locality	Alkaloid %
Seethanady	2·025	Thingale	2·034
Madamakky	1·889		
Kuntamakky (Site I)	1·661	Mavinguli	1·779
Kuntamakky (Site II)	1·917		

Soil in Coondapur Forest Division is red laterite being rich in humus at places. Temperature in summer goes high (about 40–45° C) and, in winter, drops down to 10–15° C. The area receives high rainfall of about 500 mm annually.

Coondapur region, lying between 13° and 14° N atitude, appeared to be abounding in superior chemotypes which fact is suggestive of carrying out intensive survey work in this area for collection of high alkaloid yielding plants. Such differential potencies in alkaloid content have also been reported by Wakhloo[7] and Santapau[6] indicating regional variation in alkaloid content in *R. serpentina*.

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1. Annonymous, *Indian Pharmacopoea*, 1966, p. 633.
2. Dhar, R., "Variation in alkaloid content and morphology of four geographical races of *Rauvolfia serpentina*, Benth," *Proc. Indian Acad. Sci.*, Section B, 1965, **62**, 242.
3. Dutta, R. K., Chopra, I. C. and Kapoor, L. D., "Cultivation of *Rauvolfia serpentina* in India," *Econ. Bot.*, 1963, **17** (4), 243.
4. Kazim, M., *Tour Report*, NBPGR, New Delhi 1978 (Unpublished).
5. Mital, S. P., Issar, S. C., Bhagat, N. R., Maheshwari, M. L., Kidwai, M. A. and Saxena, D. B., "Selection for superior genotypes in Serpagandha (*Rauvolfia serpentina* (L.) Benth. Ex Kurtz.)," *IDMA Bltn.*, 1976, **15**, 175.
6. Santapau, H., "Botanical aspects of *Rauvolfia serpentina*," *Symposium on R. Serpentina, Indian J. Pharma.*, 1956, **18**, 1.
7. Wakhloo, J. L., "Variation in the total alkaloid content of *Rauvolfia serpentina* roots—a consideration from ecological point of view," *J. Indian Bot. Soc.*, 1963, **42**, 215.

MICROSOMAL DEGRANULATION BY TEA TANNINS

EPIDEMIOLOGICAL surveys have recently associated a higher incidence of carcinoma with the consumption of tea as a beverage around the world[1–4]. The tannin fractions from tea (*Camellia sinensis*) are very active and produce tumours at the injection sites in 66% or more of the treated animals[5]. In view of the fact that tea tannins have not so far been studied for their

carcinogenic potentials by any short-term technique, it was decided to undertake such studies using the microsomal degranulation technique[6]. Our results demonstrate that tea tannins are potential carcinogens.

Tannins were extracted from tea leaves and the extract was lyophilized (Pradhan *et al.*[7] and Wail Me *et al.*[8]). For *in vitro* experimetns, rat liver microsomes from three rats (Kasauli strain) weighing 150–180 g each, and fed *ad libitum* were prepared[6]. Microsomes were incubated with (40 μg/ml) without tea tannins in the presence of 1 mM NADPH/double distilled water and 10% post-microsomal supernatant/ST buffer (225 mM sucrose and 25 mM Tris, pH 7·5)/calcium (8 mM final concentration) for two hours at 20° C. After incubation, the microsomes were pelleted down at 9,000 g and resuspended in ST buffer (final concentration. 5–8 mg protein per ml). For *in vivo* experiments tea tannins were injected S.C. (160 mg/K b.w.)[7] into three rats (150–180 g) fed *ad libitum*. After a lapse of 10 hrs rat liver microsomes from treated and control rats were prepared[6].

TABLE I

Degranulation of rat liver microsomes by tea tannins

Composition of incubated mixtures	Per cent loss of NA	
	RNA/protein basis	RNA/phospholipid basis
(*a*) *In vitro* studies		
Microsomes (control)	..	..
Microsomes + DDW[a] + NADPH + ST buffer[b]	5·0	5·49
Microsomes + TT[c] + DDW + ST buffer	23·08	23·91
Microsomes + TT + NADPH + ST buffer	34·31	43·47
Microsomes + TT + NADPH + PMicS[d]	0	4·34
Microsomes + TT + NADPH + Ca^{+2}	0	0
(*b*) *In vivo* studies		
Microsomes (from control rats)	..	..
Microsomes(from rats injected with TT)	33·32	34·51

(*a*) DDW — Double distilled water.
(*b*) ST buffer — 0·225 M sucrose and 25 mM tris, pH 7·5.
(*c*) TT — Tea tannins.
(*d*) PMicS — Post-microsomal supernatant.

Protein concentrations were assayed[9] using bovine serum albumin as a standard. RNA concentrations in all the samples were determined according to the modified method of Munro and Fleck[10], Phospholipids were extracted from membrane samples[11] and phosphorus estimations were carried out[12]. Phosphorus values were converted to phospholipids using a factor of 25.

Degranulation of rough endoplasmic recticulum of rats by carcinogens both under *in vivo*[13,14] and *in vitro*[15,16] conditions is now an established fact and *in vitro* degranulation has been evaluated as one of the reliable techniques for the detection of carcinogens[16].

Results presented in Table I show that the tannins degranulate rat liver microsomes over 20% and the degree of degranulation is enhanced to about 40% level in the presence of NADPH which is required for the conversion of carcinogens to electrophiles which attack the biological nucleophiles like nucleic acids and proteins. It is further clear from the data that the presence of Ca^{+2} in the post-microsomal supernatant or if added from outside protects the microsomes against degranulatory attack of carcinogens. This observation corroborates our earlier report[6]. *In vivo* administration of tea-tannins also provides micrsomes with over 30% loss of ribosomal RNA as measured both on the basis of RNA/protein and RNA/phospholipids ratios. Thus both *in vitro* and *in vivo* experiments demonstrate that tea tannins are potential carcinogens.

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1. Kumar, K. M. and Ramachandran, P., *Ind. J. Cancer*, 1973, **10**, 183.
2. Segi, M., *Gann*, 1975, **66**, 199.
3. Morton, J. F., *J. Crude Res.*, 1972, **12**, 1928.
4. Menakamt, W., Woir, C. S. and Jain, D. K., *Br. J. Cancer*, 1971, **25**, 225.
5. Kapadia, G. J., Paul, B. D., Chugh, E. B., Gosh, B. and Pradhan, S. N., *J. Natl. Cancer Inst.* 1976 **57** (1), 207.
6. Gupta, M. M. and Dani, H. M., *Ind. J. Expl. Biol.*, 1979, **17**, 1144.
7. Pradhan, S. N., Chugh, E. B., Gosh, B., Paul, B. D. and Kapadia, G. J., *J. Natl. Cancer Inst.*, 1974, **52**, 1579.
8. Wall Me, Taylor, H., Ambrosia, L. and Davis, K., *J. Pharm. Sci.*, 1969, **58**, 839.
9. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 195', **193**, 265.
10. Munro, H. N. and Fleck, A., *Analyst* (*Lond.*), 1966, **91**, 78,

11. Folch, J., Lees, M. and Sloane-Stanley, G. H., *J. Biol. Chem.*, 1957, **226**, 497.
12. Ames, B. N., *Methods in Enzymology*, (Academic Press, Inc., New York), 1966, **8**, 115.
13. Butler, W. M., *Amer. J. Pathol.*, 1966, **49**, 113.
14. Gustafsson, R. G. and Afzelius, B. A., *J. Natl. Cancer Inst.*, 1963, **30**, 1045.
15. Williams, D. J. and Rabin, B. R., *Nature* (*London*), 1971, **232**, 102.
16. Purchase, I. F. H., Longstaff, E., Ashby, J. Styles, J. A., Anderson, D., Lefevre, P. A. and Westwood, F. R., *Br. J. Cancer*, 1978, **37**, 873.

RED-CELL 2,3-DIPHOSPHOGLYCERATE AND POTASSIUM HOMEOSTASIS IN THYROID DISORDERS

In a recent issue of your journal, Sarkar *et al.*[1] report the finding of a signiacant rise in the potassium content of plasma and red cells, with simultaneous increase in the erythrocyte 2, 3-diphosphoglycerate (2, 3-DPG) concentration in hyperthyroid rats.

In 21 female patients, aged between 18 and 67 years (mean ± SD : 48 ± 14 years), with ciinical as well as hormonal evidence of hyperthyroidism, (whom we studied on 29 occasions), we also found abnormally high levels of 2,3-DPG, which could not be accounted for by alterations in the haematocrit (Hct), haemoglobin (Hb) or serum inorganic phosphate (P) levels[2]. The 2 3-DPG increase however, did show in our cases a significant correlation ($p > 0{\cdot}05$) with the serum thyroid hormones (serum total T3 and T4). Hct, Hb and P were determined by standard haemotological techniques, while determinations of red cell 2,3-DPG were performed by Eaton *et al.*[3] modification of Bartlett's[4] chromotropic acid (4,5-dihydroxy-2,7-naphthalene-disulfonic acid) method. Hormone concentrations were measured by means of the Abbott Laboratories radioimmunoassay kits (normal ranges : 100–210 ng/dl and 5–13 μg/dl for T3 and T4 respectively). Although we did not include the estimates of the plasma potassium content (K) (determined photometrically) in the mentioned work, at the same time as our 21 hyperthyroid patients were analysed for 2,3-DPG, Hct, Hb, P, T3 and T4 levels an analysis of K was made for 16 or them, using the same blood samples. Our findings in these 16 patients as well as those in euthyroid control subjects are shown in Table I. Increased levels of 2,3-DPG (6·01 ± 0·78 mM) are present in these female patients and show a clear statistical difference with the levels in the euthyroid control series ($p > 0{\cdot}001$). In both groups. however, K appears to bel argely similar and within normal limits. We have also been unable to establish any relevant correlation between the red-cell, 2,3-DPG concentration and the potassium content of plasma.

TABLE I

Haematological and thyroid function data in patients with hyperthyroidism and in euthyroid controls. Values shown are mean ± SD with SEM in brackets.

	Hyperthyroid (N = 16)	Euthyroid (N = 32)
Age (years)	48±14 (3·6)	51±14 (2·5)*
Hb (g/dl)	13·6±1·4 (0·3)	14·0±0·8 (0·1)*
Hct (%)	40·8±5·4 (1·3)	41·7±2·9 (0·5)*
P (mg/dl)	3·2±0·4 (0·1)	3·4±0·5 (0·08)*
T3 (ng/dl)	327±126 (32)	139±26 (4·6)**
T4 (μg/dl)	16·7±6·3 (1·6)	9·3±1·7 (0·3)**
K (mEq/1)	4·24±0·33 (0·08)	4·20±0·33 (0·05)*
2,3-DPG (mM)	6·01±0·78 (0·2)	4·89±0·50 (0·08)**
2,3-DPG μmoles/g Hb)	17·9±2·5 (0·6)	15·0±1·5 (0·2)**

(N = number of experiments; * not significant; ** $p < 0{\cdot}001$).

Our results in human patients are not in agreement with those reported by Sarkar *et al.*[1], regarding the homoestasis of potassium in hyperthyroid situations. In any event, it should be borne in mind that the findings of the authors mentioned may not be entirely at variance with ours, owing to the induced nature of the disorder and the fact that male rats were used whereas our subjects were all women, who had developed the hyperthyroid state spontaneously.

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1. Sarkar, S. R., Singh, L. R., Banerji, R. and Chaudhuri, B. N., *Curr. Sci.*, 1979, **48**, 880.
2. Alvarez-Sala, J. L., Urbàn, M. A., Sicilia, J. J., Diaz Fdez, A. J., Fdez Mendieta, F. and Espinós, D., *Acta Endocrinol.*, 1980, **93**, 424.
3. Eaton, J. W., Brewer, G. J., Schultz, J. S. and Sing, C. F., In *Red Cell Metabolism and Function*, Brewer, G. J., Plenum Press, New York, 1970, p. 21.
4. Bartlett, G. R., *J. Biol. Chem.*, 1959, **234**, 469,