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Effect of 5,5-diphenylhydantoin on carotenoid pigments in human serum using laser Raman spectroscopic technique

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We have applied the laser Raman spectroscopic technique to demonstrate the side effect of the anticonvulsant drug (5,5-diphenylhydantoin) on the human serum. It has been found that the drug–plasma interaction induces significant changes in the intensity ratio of β-carotenoid bands. This suggests that the plasma-bound carotenoids may be involved in drug–plasma interaction. Repetitive measurements on the serum of both normal controls as well as grand mal epileptic cases undergoing anticonvulsant drug therapy showed that, apart from the three maxima at 1535, 1162 and 1010 cm^{-1}, additional three weak bands of β-carotene were detected at 980, 1193 and 1220 cm^{-1}. These newly detected Raman bands were not seen to be affected as a result of 5,5-diphenylhydantoin drug therapy.

THE Raman spectroscopic technique is an excellent method for examining the vibrational energy levels of biomolecules, which are sensitive to the chemical nature of the constituent groups and intermolecular interactions[1]. Studies on carotenoids in blood plasma and their direct relationship to diseases using Raman spectroscopy have been carried out by Larsson and Hellgren[2]. Rein *et al.*[3] detected and identified carotenoid pigments in human blood plasma using resonance Raman technique. Extensive work on the triplet state of all-*trans*-β-carotene using Raman spectroscopy has been reported by Jensen *et al.*[4]. Recent biological and biomedical applications of Raman spectroscopy include the study of membrane-bound proteins[5] and identification of phosphate-type kidney stones.[6] Several biophysical and biochemical studies have been carried out on the plasma levels of anticonvulsant drugs which are seen to be bound to plasma proteins. Phenytoin (5,5-diphenylhydantoin), the anticonvulsant drug (Figure 1), is known to displace the normal protein-bound substances from their binding sites.[7] Manfait *et al.*[8] have applied the Raman effect to the study of drug–DNA interactions.

The present study indicates that although Raman spectroscopy of grand mal epileptic cases undergoing anticonvulsant drug therapy (5,5-diphenylhydantoin) over a prolonged period {$(GME)_p$} and normal controls (NC) are similar in some respects, there are significant intensity ratio variations, which will be discussed later. During investigation of drug–plasma interactions, three additional β-carotenoid peaks were detected corresponding to 961 cm^{-1}, 1193 cm^{-1} and 1220 cm^{-1}. Thus we have succeeded in showing that the changes in the relative intensities of β-carotenoid bands in human serum studied by Raman spectroscopy provide additional insight in understanding the biochemistry of the anticonvulsant drug–plasma interaction.

Sera of NC and $(GME)_p$ obtained by centrifugation from several samples were subjected to laser Raman spectrophotometer (Jasco, Model NR-1000) consisting of a photomultiplier unit (R-464) and an argon-ion laser at 488 nm, 80 mW having a scan speed of 5 cm^{-1} s^{-1}. An average of 4 scans was taken by the computer. Sensitivity was 5x kpps × 100 pA. Time constant was 0.5 s. Zero suppression was at 2.8. Interference filter was used. ν expansion was at 10 cm^{-1} div^{-1}.

The following characteristics have been observed in a typical spectrum (Figure 2) of NC serum: (a) strong Raman bands at 1534 cm^{-1} (ν_6) and 1160 cm^{-1} (ν_3) corresponding to C=C and C–C stretching vibrations respectively due to carotenoids and a moderately strong band at ~ 1010 cm^{-1} (ν_2) corresponding to C–CH_3

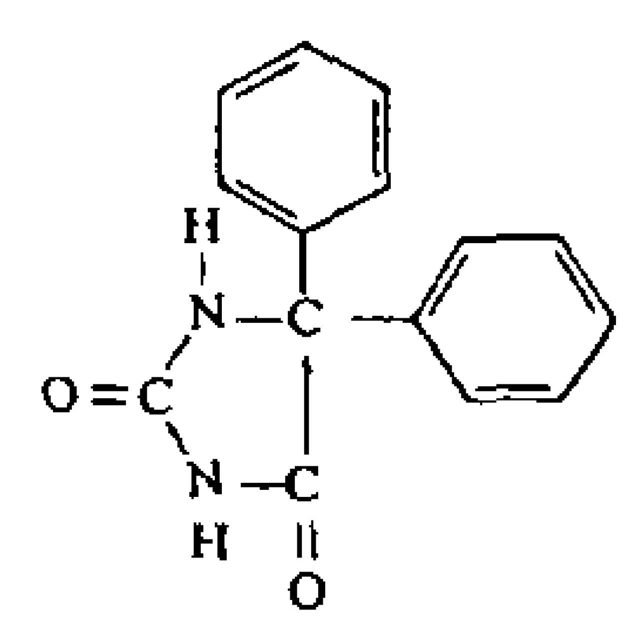

Figure 1. 5,5-diphenylhydantoin.

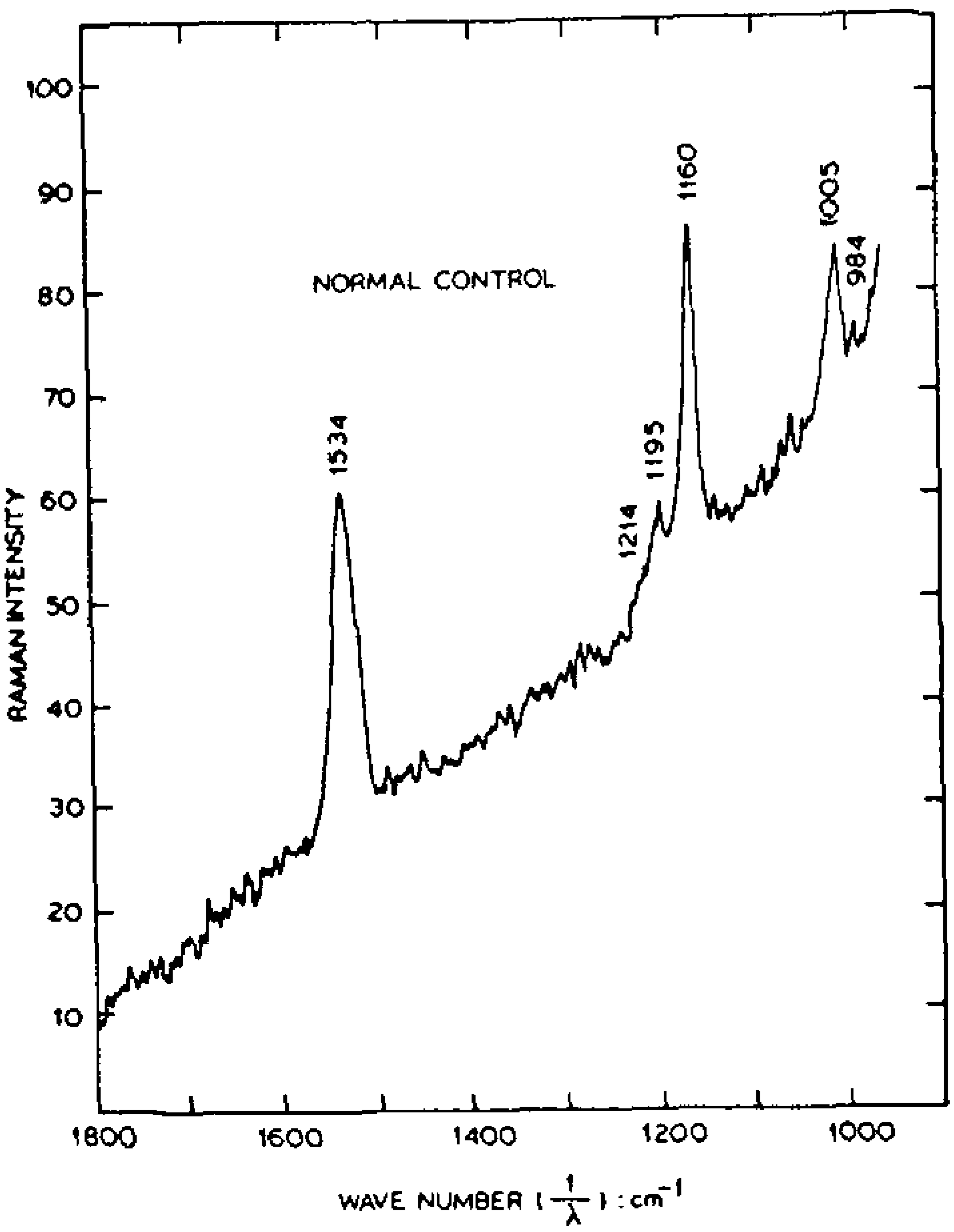

Figure 2. A typical Raman spectrum from a healthy individual (normal control).

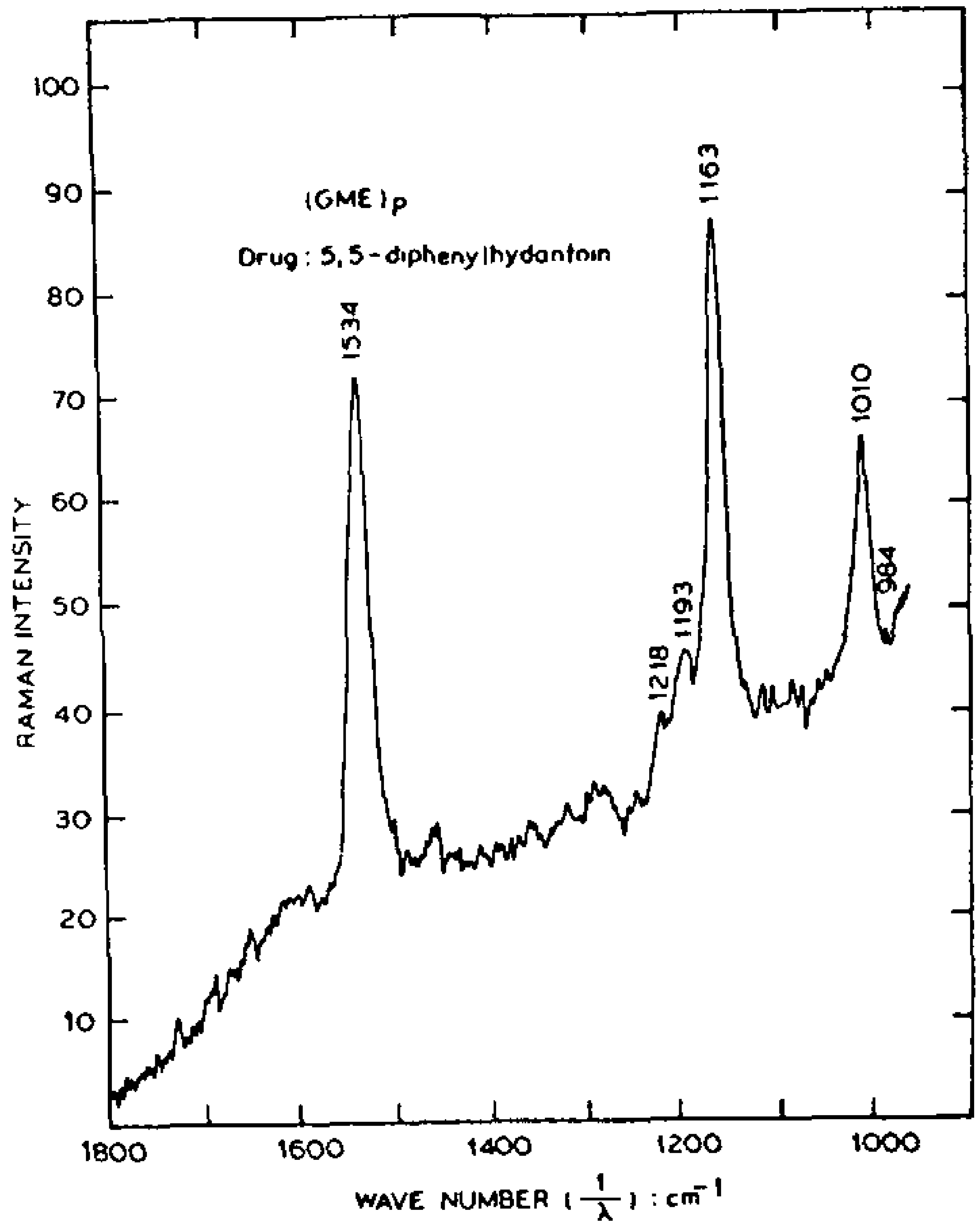

Figure 3. Raman spectrum from a grand mal epileptic patient undergoing 5,5-diphenylhydantoin drug therapy.

stretching vibrations from aromatic protein side chains and/or C–H in plane bending mode, (b) additional three peaks of *trans*-β-carotene at 984 cm^{-1} (ν_1) (very weak) described as in plane angular deformation of C–C–C and C–C–H, 1193 cm^{-1} (ν_4) (weak) and 1218 cm^{-1} (ν_5) (very weak) due to contributions from C–C stretching and C–C–H in plane deformation as well as some C=C stretching. All $(GME)_p$ spectra, within experimental errors, exhibit the same general characteristic features as those of NC as seen in Figure 3. However, significant variations in terms of intensity changes in $(GME)_p$ spectra when compared to NC are noticed. The band at 1010 cm^{-1} (ν_2) is taken as an internal reference. The ratio $I\nu_3/I\nu_2$ and $I\nu_6/I\nu_2$ for NC and $(GME)_p$ has been calculated and summarized in Table 1. A significant increase in the intensity ratio is observed in $(GME)_p$. This increase can be explained in terms of changing bond order, which may be attributed to perturbation by treatment with 5,5-diphenylhydantoin.

In continuation of the earlier work by Rein *et al.*[3] on blood plasma investigations by Raman spectroscopy, we have been able to detect three additional Raman bands of *trans*-β-carotene, which, to the best of our knowledge, have not been reported yet. From the above study, we conclude that the side-effects of 5,5-diphenylhydantoin (or any other anticonvulsant drug) can be studied by monitoring the intensity ratio of various Raman bands.

Table 1. Comparison between the spectral intensity ratio of NC and $(GME)_p$

Nature of sample	Intensity ratio $I\nu_3/I\nu_2$	$I\nu_6/I\nu_2$
NC	1.03	0.72
$(GME)_p$	1.32	1.10

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A new hybrid between *Sesamum alatum* and *Sesamum indicum*

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An investigation was taken up to study the possibility of incorporating resistant genes of phyllody disease caused by mycoplasma-like organisms to the cultivar of *Sesamum indicum* ($2n=26$) from the wild species *S. alatum* ($2n=26$), and the results are reported here. A successful hybrid between *S. alatum* and *S. indicum* was synthesized. The hybrid was characterized based on its distinct morphological features, pollen fertility, meiotic pairing and separation. Study of F_2 and back-cross progeny are in progress.

SESAME (*Sesamum indicum* L.) is an ancient oil-yielding crop cultivated extensively in Tamil Nadu. There is considerable yield loss every year due to the incidence of phyllody disease caused by mycoplasma-like organisms (MLO). The resistance to phyllody was reported in the wild species *S. alatum* ($2n=26$) through artificial screening[1]. Earlier attempts to transfer this trait from wild to the cultivars were not successful[2,3].

Seeds of *S. indicum* cultivar CO 1 and *S. alatum* were used for the study. Seedlings of parents were raised in raised beds. Flower buds, one day prior to opening, were emasculated during evenings and bagged. Crossing was done by collecting pollen from the desired male parent between 7.00 and 7.30 a.m. Crossed flowers were covered with muslin cloth to avoid contamination. Number of flowers crossed and the capsule set were recorded. Seeds from the few capsules obtained from the crosses were raised in pots to study the F_1 hybrid performance. Morphological observations were followed by cytological investigations. To study meiosis, young flower buds were fixed in Carnoy's fluid at 11 a.m. and pollen mother cell smears were made using one per cent acetocarmine. Photomicrographs were taken using 20 ASA film with 'Pentax' spotmatic camera.

The crossability was very low between *S. alatum* and *S. indicum* whereas there was no capsule set in the reciprocal cross. Of 1102 flowers pollinated, only 0.04% capsule set was noticed, and only one crossed seed germinated. The hybrid was morphologically distinct from either of the parents (Figure 1, Table 1). The distinguishing morphological features of this hybrid were trifoliate leaves, violet-coloured flowers, variegated capsules, 2–4 purple nectary glands on either side of the petiole and wingless seeds. The hybrid showed 82–85 per cent pollen fertility with normal capsule set.

Meiotic studies of parents and hybrid revealed regular bivalent (13 II) formation at diakinesis and Metaphase I. Higher associations were absent (Figure 2). The PMC's at Anaphase-I and II showed normal separations and formation of quartets. The hybrid exhibited normal course of meiosis, high degree of chromosome homology and high pollen fertility. This may be attributed to intergenomic homology between the chromosomes of the two parental species having the same chromosomal status ($2n=26$). Though earlier attempts failed to produce viable hybrids in this cross combination[2,3], Joshi[4] presumed that there might be an early abortion

Table 1. Morphological characters of parents and hybrids

Characters	*S. alatum*	*S. indicum*	Hybrid
Leaf shape	Tri-, tetra-, penta-lobed, linear and entire leaves	Linear and entire leaves	Tri-lobed, tri-partite, long and linear control leaflet
Colour of the flower on the day of blossoming	Maroon	White	Violet
Glands	1 or 2 purple glands	Yellow glands (simple)	2–4 purple glands
Pigmentation of stem	Green with slight purple wash	Green	Green with slight purple wash
Capsule	Deeply grooved, cylindrical capsules tapering towards base with forked tip	Four to six loculed, short hairs all over the fruit	Deeply grooved, cylindrical, capsule with forked tip variegated
Seed	Winged, dark brown, rough	Wingless, black smooth	Wingless, dull brown, rough