After obtaining the monolayer, cells were passaged to new flasks by trypsinization. The old medium was removed and replaced with 1 ml of 0.025% trypsin-EDTA. The enzyme activity was arrested by adding 1 ml of FBS after a minute. The detached cells along with FBS were centrifuged at 1000 rpm for 10 min. The pellet was then suspended in MEM by gentle tituration and the contents transferred to a tissue culture flask for incubation. Alternatively, the monolayers were also harvested by scraping, employing a sterile rubber policeman and passaged to a new flask in MEM.

The trypsinized cells attached to flasks in clumps and individually, retaining the original fibroblast-like shape. Cells were in good condition for about a week, after which they died. The cells that were detached using a sterile rubber policeman appeared in clumps and attached well in the new flask. These clumps of cells showed growth along the periphery. Attachment of cells and monolayer formation was better with harvesting by scraping than with trypsinization. After harvesting the cells (either by scraping or trypsinizing), the few pieces of tissue and undetached monolayer in the original flask resumed normal cell growth and formed monolayer. Some tissue pieces in the original flask showed emerging cells which did not spread away from the explant; rather they stayed close to the tissue and formed a network.

In this study, a successful primary culture could be obtained using only the synthetic medium (MEM) supplemented with 15% FBS without using muscle extract. Homologous fish muscle extract has been used for successful development of primary cultures[4,6].

In general, growth and development of cell monolayer from heart tissue explants were good and easy to maintain. Besides, heart tissue of Indian major carp was found ideal for cell culture, as it is easy to collect aseptically and also it is contamination-free compared to other visceral organs. Hence, there is scope and prospect for development of cell line from heart tissue of carps. Work on the establishment of heart cell line from the primary cultures and its characterization is under progress.

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Microgravity changes associated with continuing seismic activities in Koyna area, India

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High-precision gravity measurements were carried out during June–August, 1995 in Koyna area, India, which is known for its high intraplate seismic activities. Daily observations were recorded at four stations with respect to a reference station to monitor the possible gravity changes arising due to crustal deformations associated with seismic activities. Significant relative gravity changes (15–30 μgal) of almost same pattern were observed at different stations which is 2–3 times of the observational error. A good correlation was noticed between the relative change in the gravity field from one station to the other and with the occurrences of tremors of magnitude 2–3 which are approximately twenty in number during this period. In most of the cases (18), the relative gravity field first increases and then decreases when the seismic events take place. The observed changes in gravity field seem to be associated with seismic activities, and may be attributed to mass redistribution or seismogenic deformations such as opening of the cracks, etc. in processes taking place during an earthquake. However, the phenomenon is not fully understood and needs more such experiments over longer duration to be conducted in different geological/tectonic settings to understand its actual nature and the mechanism.

TEMPORAL variations in local gravity field over a small time period can occur due to deformation of crustal rocks in the form of mass re-distribution and change in elevation[1]. The study of variation in the gravity field due to such deformations has been made possible with the advent of high precession gravimeters which measure the variations in gravity field up to microgal levels. Hence, repeat gravity observations using such instruments help to study both aseismic and co-seismic deformations and mass redistribution. For example, the measure of vertical aseismic deformation in response to loading due to La-Grande-2 reservoir, Quebec has been

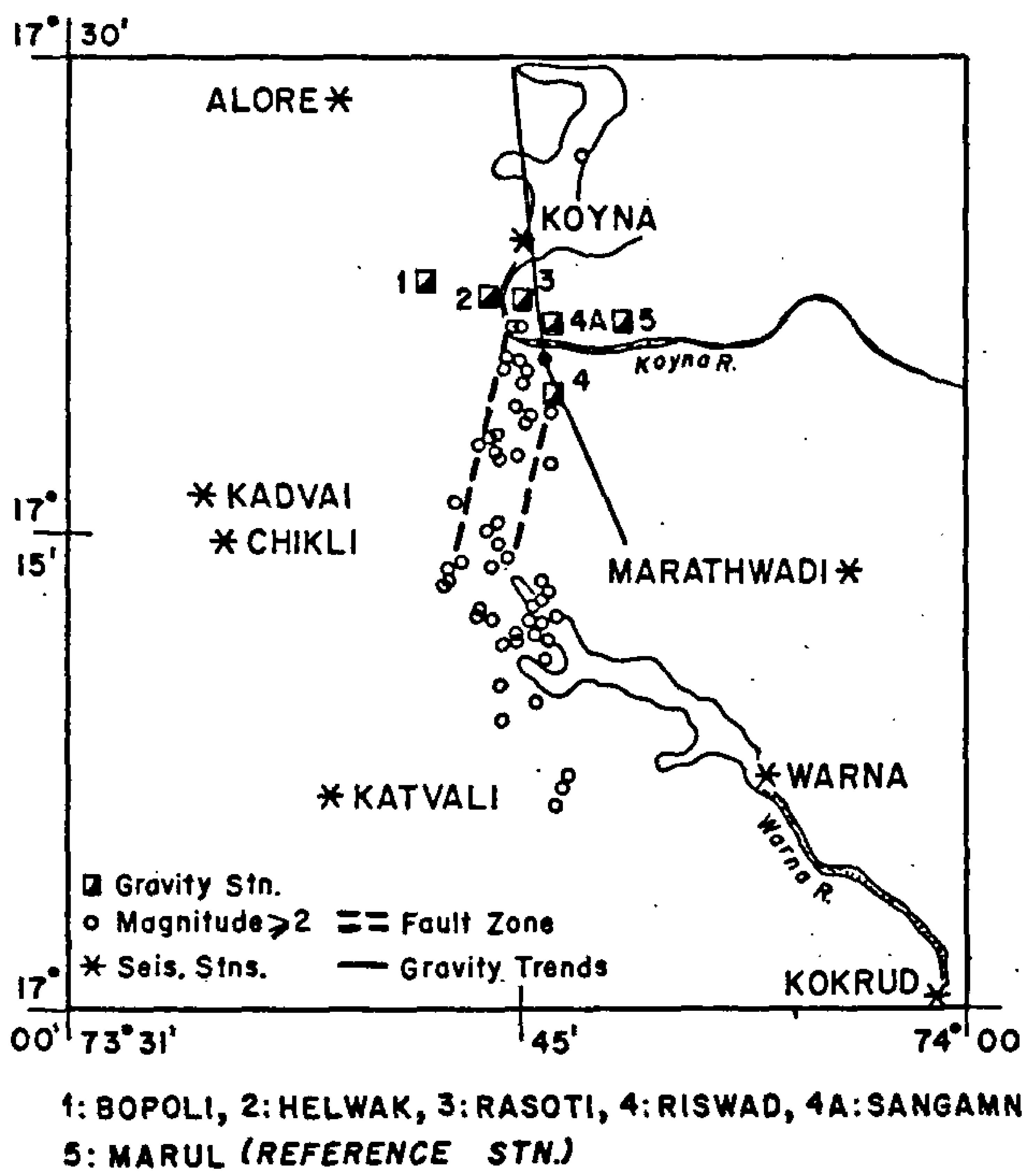

Figure 1. Locations of gravity stations and epicenters of tremors of $m \geq 2$ during 10 June–16 August.

studied using repeat gravity observations[2]. Variations of the gravity field were modelled to correlate the gravity change with mass movement due to change in the seasonal groundwater level[3]. Systematic gravity measurements in conjunction with other observations made before and after the Tangshan Earthquake of 1976, revealed dilatational process in the epicentral area[4]. Many works revealed the importance of repeat gravity measurements to study the seismogenic deformations[5–10]. Changes in gravity field are reported in some regions of seismic activities in India by Arur *et al.*[11] and Kayal *et al.*[12].

The Koyna earthquake (*M* 6.2) on 10 December 1967 had shattered the long-held view that peninsular India was seismically stable. This region has attracted attention due to continued seismic activity since the impoundment of Koyna reservoir in 1962 (ref. 13). An average of 5 to 10 earthquake of $M > 4$ and several of $M > 3$ are reported to occur every year in this region[14]. This area, therefore, provides a natural laboratory to study the earthquake processes and their effects on various physical parameters. A local digital seismic network established in this area, continuously provides the details of earthquakes occurring in this region. In the present work, gravity observations were made at five permanent stations, for more than two months. The objective of this study was to observe changes in the gravity field, if any, and investigate whether the observed change is associated with seismic activities.

The Koyna region is covered with Deccan basalts which are considered to be erupted during late Cretaceous[15]. Since this area is totally covered with trap, the underlying geology and tectonics has been a matter of wide speculation. A significant geomorphological observation in this area is the similarity of escarpment trends as observed along the continental divide and the course of Koyna river (N–S) which turns eastward near Koyna (Figure 1). It is suspected that the Koyna river itself flows along a fault[16]. After the 1967 Koyna earthquake, various geophysical studies[17–20] were carried out to decipher the major tectonics of this region. The Bouguer anomaly map of this area[17] grossly indicates a sharp gradient, almost follows Western Ghats (i.e. N–S) up to Koyna where it changes to a NNW–SSE trend, (trend marked in Figure 1) which is indicative of some structural feature. Later Deep Seismic Sounding studies[18] have delineated a NW–SE basement fault near Koyna coinciding with the Bouguer anomaly pattern. Based on seismicity trends in this region, Gupta *et al.*[21] inferred a N–S oriented fault coinciding with 73°45' longitude. However from the study of composite fault plane solutions of seismic events in this area, Rastogi and Talwani[22] suggested NW–SE and NNE–SSW as the possible strike directions of the siesmogenic fault(s). It, therefore, appears that the area might contain one or more hidden fault(s) and fractures below the trap.

Based on the empirical relations between magnitude of an earthquake and the effective distance over which gravity changes are noticeable[23] and precursor time[24], for $M > 2$ earthquake, five permanent stations (Bopoli, Helwak, Rasoti, Sangamnagar, Riswad) (Figure 1), are established in the Koyna area. Stations are marked with paint on concrete ground in a covered shed away from the road, to avoid any undesirable influences on observations and to be reoccupied to an accuracy of a centimeter. The locations of stations were planned such that two stations lie on either side of the inferred fault[21] in this region. One station (station no. 5) was used as a reference station (Figure 1). One of these five stations (station no. 4) could not be occupied after twenty days because of inaccessibility due to heavy rain and was therefore shifted to Sangamnagar (station no. 4A). At a station at Bopoli the western side was operated only during the last sixteen days due to logistic problems (Figure 1). Gravity measurements were made using LaCoste Romberg D-type gravimeter (D-116) with electronic read out, which gives a dial reading of approximately 1 μgal.

The following measures were adopted in order to increase the observational precision.

- A–B–C–A–B–C survey procedure is followed so that every station is occupied twice in a loop in interlocking loop system (Figure 2).

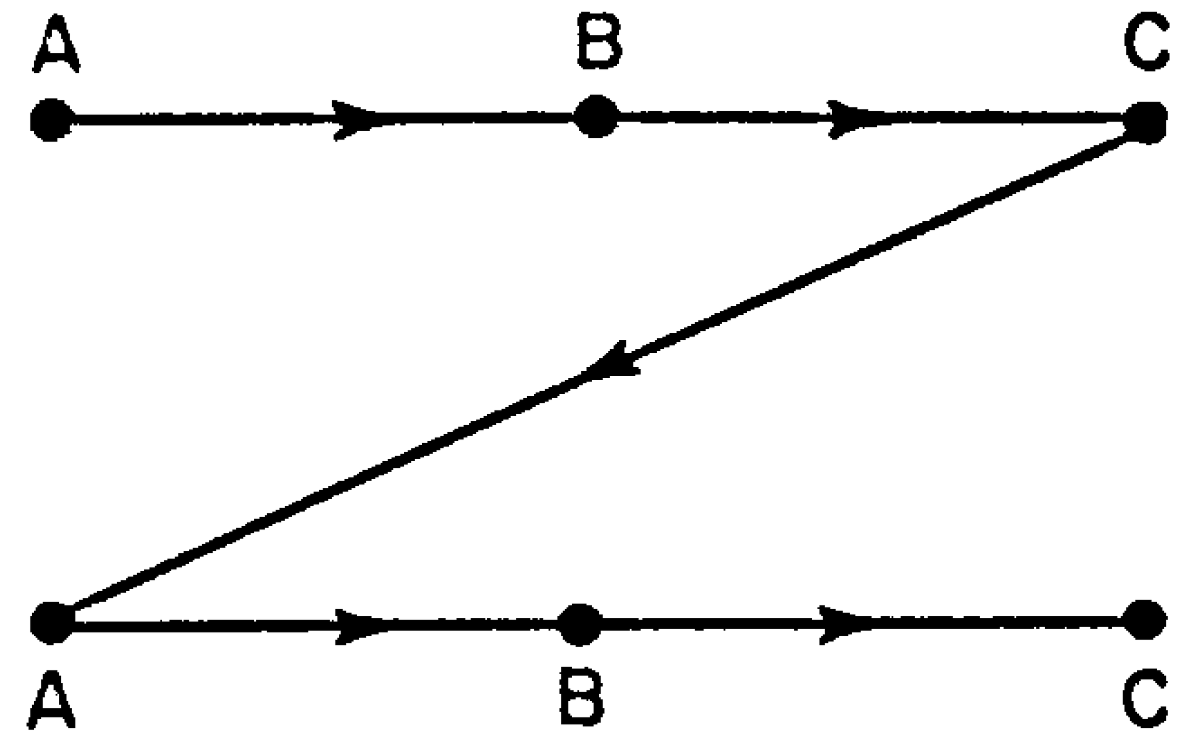

Figure 2. Schematic diagram of recording gravity stations in loop.

- The road distance between stations is chosen almost equal, to minimize systematic error due to variable driving time.
- Each point was reoccupied within one hour so that even small drift of the instrument can be corrected.
- During observations the instrument was kept in the same direction at a station in order to eliminate any directional influence on the observations.
- The entire profile was completed in 3–4 h, recording at least two readings at every station.
- The same setting of the gravimeter was used throughout the survey to avoid any error arising from resetting of the instruments.
- Readings were taken until three consecutive readings were obtained within 1 dial reading.

Tidal correction was made using the standard program GRAVPACK[25] to depict non-tidal variation of gravity. Drift corrections were made assuming linear drift for one hour, which is found quite satisfactory based on static drift recorded both in the field and at NGRI, Hyderabad. Pressure correction was not applied considering that it will be of the order of one microgal[26]. Further, loop error is estimated by taking the average difference between stations in a loop and distributed over all the stations in a loop. The loop closure errors, i.e. A–B + B–C = A–C are usually found to be less than 3 μgal. This combined with other possible random errors may amount to a maximum of about 5 μgal, which is much less than the observed variation of 15 to 30 μgal. The expected observational error was obtained by conducting a similar experiment in a controlled environment at Hyderabad where a random error of 5–10 μgal was noticed between daily observations at a particular station. It is unlikely, therefore, that the recorded variations are related to any observational random error as discussed by Torge and Kanngieser[27]. Finally the means and standard deviations of average difference between reference station and stations, after applying loop closure error, were computed (Table 1).

Table 1. Means and standard deviations of average differences after applying loop closure errors

Station no. (Figure 1)	Mean (mgal)	Standard deviation (μgal)
1	0.577	8.0
2	1.2987	9.0
3	0.2807	6.6
4	0.7092	7.2
4A	8.2532	7.5

Several models have been proposed to explain the temporal variation of the gravity field[9,28–30], which are basically related to fault dislocations or dilatation of a focal body.

(i) Change in gravity field has been explained according to the static fault dislocation model[9,10]. Gravity change caused due to strike-slip dislocation of rectangular fault can be collected in Cartesian coordinate system as follows:

$$g(x, y) = \alpha GU[-q \sin \delta / R + q^2 \cos \delta / R(R + \Phi) - \beta/(2\pi)\{-d_1 q/[R(R + \Phi)] - q \sin \delta/(R + \Phi) - I \sin \delta\}]\|. \qquad (1)$$

Chenery notation '‖' is used to represent the substitution.

$$f(\theta, \Phi)\,\| = f(x, p) - f(x, p - w) - f(x - l, p) + f(x - l, p - w),$$

where $I = (1-2\sigma)[\log (R + d_1) - \sin \delta \log (R + \Phi)] \sec \delta$, $R^2 = (\theta^2 + \Phi^2 + q^2)$, $q = y \sin \delta - d \cos \delta$, $d_1 = \Phi \sin \delta - q \cos \delta$ and if $\cos \delta = 0$, $I = -(1-2\sigma)q/(R + d_1)$.

In these formulae, G is the gravitational constant, U the strike-slip component of arbitrary fault dislocation, l the half length of the fault, d the depth of the fault, δ the dip angle of the fault, α the density, $\beta = 0.3086$ gal/cm free air gradient, σ is the Poisson ratio (= 0.25).

(ii) Ruihao and Zhaozhu[4] have examined the gravity change due to stress condition of focal body during earthquake cycle based on the dilatancy model. In the initial stages, stress accumulation causes increase in density which will increase the gravity field. A vertical cylinder as focal body in a stress state gives rise to a gravity field as:

$$g = 2\pi\alpha G[l + r - \sqrt{(l^2 + r^2)}][(1 - \sigma)(1 + 2\sigma)]\,(\text{stress}/E). \qquad (2)$$

If preparation zone is considered along fault, it is more appropriate to consider a horizontal cylinder instead of a vertical cylinder. In such a condition, the above equation leads to the following expression by

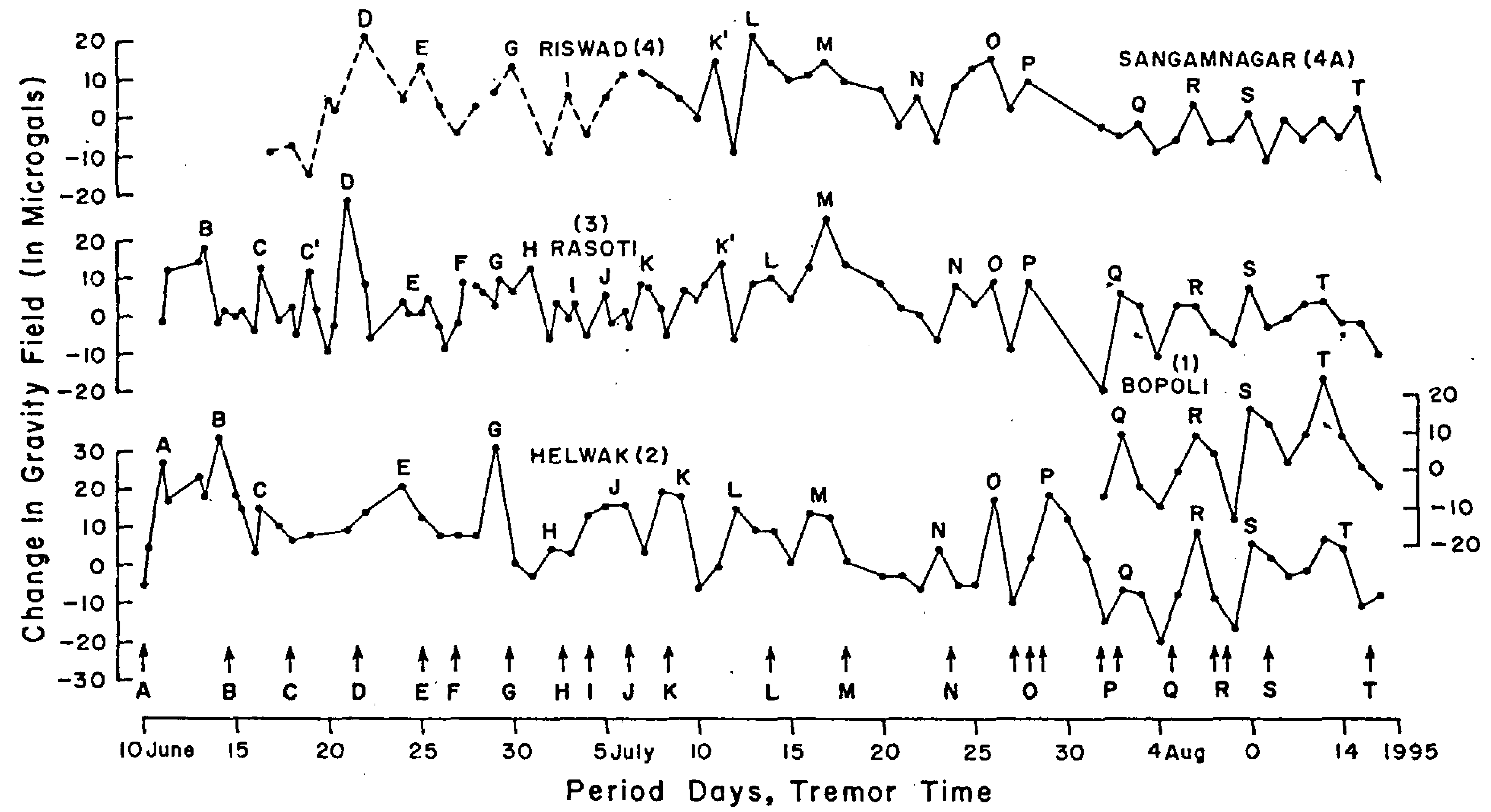

Figure 3. Temporal variation of gravity field from 10 June to 17 August at various stations with reference to station no. 5 (Marul), along with occurrences, time of tremors ($M \geq 2$) in Koyna area (north of 17°15') and three tremors ($M \approx 3$) in Warna area (south of 17°15').

substituting the geometrical factor of horizontal cylinder.

$$g = \pi \alpha G r^3/(r^2 + x^2)[1/\sqrt{\{1 + (r^2 + x^2)/(y + l)^2\}} - 1/\sqrt{\{1 + (r^2 + x^2)/(y - l)^2\}}][(1 - \sigma)(1 + 2\sigma)] (\text{stress}/E), \quad (3)$$

where l, r are half of the length and radius of cylinder, σ is the Poisson ratio, E is the Young's modulus, x, y are the coordinates of point and α is the density.

The differences in the gravity field at each of the stations with respect to the reference station (Marul) are computed after accounting for tidal, drift and loop closure errors. These differences were adjusted to the mean value and the representative plot of variations of adjusted difference at various stations is shown in Figure 3. Significant variations in relative gravity field are observed (15–30 μgal) which is 2–3 times more than expected random (observational) error (5–10 μgal) and standard deviations (Table 1). The time of occurrences of tremors of $M \geq 2$ in Koyna area (i.e., north of 17°15', Figure 1) and three tremors of $M \approx 3$ in Warna area (south of 17°15', Figure 1) (*Bulletin MERI*[31,32]) are indicated by arrows in Figure 3. It appears from Figure 3 that relative gravity changes are correlatable from one station to the other and with the occurrence of seismic activities. These changes in gravity field are approximately 15–30 μgal. There are approximately twenty seismic events of magnitude 2–3 numbered as A to T in Figure 3. Corresponding to most of these seismic events, variations in gravity field with some differences in amplitude are recorded at different stations as marked in Figure 3. There are three plots of daily variation in gravity field for almost full period from 10 June to 17 August 1995 corresponding to stations at Helwak, Rasoti and Riswad/Sangamnagar. At Bopoli, the gravity observations were made only during August 1995 and therefore the gravity variation plot for this station is confined only to this period. A close examination of these plots (Figure 3) suggests change in gravity field correlatable to the seismic tremors marked at the bottom of this figure. Accordingly, the changes in the gravity field in these plots are also named as A to T corresponding to the seismic events marked using the same notation.

The observations plotted in Figure 3 can be broadly characterized into three groups (Table 2) which suggests that:

i) All the events (20) are characterized by relative change in the gravity field at least at one station.
ii) Change in gravity field is consistent at all the stations with occurrence of fourteen events.
iii) In all the cases, the relative gravity field first increases and then decreases. The seismic events in 18 cases are associated with the decreasing trend in

gravity, and in only two cases, A and F, which are not very clear, it has taken place when the field is increasing. However this pattern may change if there is any change at reference station itself.

iv) The changes in relative gravity are more significant at Helwak and Bopoli compared to Sangamnagar, Riswad and Rasoti. This could be because of their locations on either side of inferred fault. A similar observation has been noticed by Akin *et al.*[33].

v) There are some cases of change in gravity field at some stations (C' and K') which might be due to some other unknown causes. But they are not consistent from one station to the other.

vi) The amplitude of the variation in the gravity field differs considerably from one station to the other and is sometimes shifted in time. This might be due to the differences in the time of observation and the occurrence of seismic tremors or the location of different stations *vis-à-vis* epicenter of the earthquake.

If we consider the models where change in gravity field is accompanied by a change in elevation (2–3 μgal/cm), an elevation change of 8 to 12 cm is required to explain the observed variations in gravity field (15–30 μgal). Frequent changes in elevation of this order over a small time period are very unlikely and therefore this model cannot satisfactorily explain the observed changes in the gravity field. However, changes in gravity field do not necessarily follow a unique relation with a change in elevation[34,35]. The other source of variation is subsurface mass redistributions. However, it is a complicated problem to resolve redistribution of mass with microgravity measurements alone in an area subjected to both, change in groundwater regime and seismogenic deformations. In the present case, order of gravity variations is almost the same before and after the monsoon. The effect of commencement of monsoon (21 June) which raised water level in reservoir and groundwater level in a well located between station no. 2 and station no. 3, has not been noticed in the nature of gravity variations (Figure 3). Further there is no pumping well in the vicinity of any station which can appreciably change the water table. The study of fluctuation of water level is being carried out in various bore wells[36]. Observations of water level change in two wells in this region, one located near gravity reference station and the other in between station no. 2 and station no. 3 show less than 10 cm of relative variation (I. Radhakrishna, pers. commun.). This magnitude of change in water level can produce a variation of hardly 4 to 5 μgal in gravity field[3] which is much less compared to the observed variations. Moreover, changes in gravity field due to changes in water level cannot be same at all the stations which are far apart as in the present case. Hence it implies that the observed gravity changes may not be related to the changes in the water level. Since observed gravity changes cannot be explained by either change in elevation or change in groundwater level, it appears to be caused by the redistribution. Subsurface mass at greater depth may be arising due to seismic activities.

In order to find out the possible cause of the gravity changes recorded in present case, the two models described above are considered.

Composite fault plane solution of seismic events of this area has revealed left lateral vertical strike slip motion with the strike direction being NNE–SSW[22]. To model the observed gravity changes in fault dislocation model, the fault parameters are chosen based on the spread and focal depth of earthquakes in this region. As the distance between Koyna and Warna (Figure 1) where the epicenter is located is 40 km and maximum reported focal depth is 5–10 km, the length and width of presumed fault are taken as 40 km and 10 km respectively. The fault angle is taken as 90 degrees because of their nature being vertical strike slip. With these parameters, eq. (1) yields strike slip dislocation up to 50 cm for an observed variation of 15–30 μgal in the gravity field, which does not seem to be feasible. The change which is dynamic in nature, therefore, cannot be explained with static fault dislocation model.

The observed variation in the present case which follows the same pattern as reported for Tangshan Earthquake of 1976 (ref. 4) should therefore be explained as build up and release of stress causing mass redistribution. In the present case almost the same gravity change is observed at a station located nearer to the epi

Table 2. Categorization of events

Events occurring during decrease in gravity field		B, C, D, E, G, H, I, J, K, L, M, N, O, P, Q, R, S, T.
Events occurring during increase in gravity field		A, F.
Unclear signatures/extra signatures	Helwak:	D, F, I.
	Rasoti:	G/C', K'
	Riswad/ Sangam Nagar:	F, J/K'

central area and at the other station away from it. Hence a horizontal cylinder model along the inferred fault is preferred over the vertical cylinder to explain the change in gravity field (≈ 25 μgal). The following parameters of the assumed horizontal cylinder along the fault are used. Length = 40 km, radius = 10 km, density = 2.8, Poisson ratio = 0.25, Young's modulus = 8×10^{11}, stress = 8×10^{7}. Although these parameters can explain the change in gravity field which is almost equal to the observed one, it remains enigmatic that such a big area would be involved for $M \approx 2$ earthquakes. These two models do not satisfactorily explain the observed changes and thus there is a need to think for other possible explanations.

Groten and Becker[37] have reported changes in the gravity field which are not accompanied by any horizontal or vertical position change using G.P.S. measurements and Akin *et al.*[33] also did not find any correlation between strain and gravity change in North Anatolian Fault Zone (NAFZ) Turkey. A similar situation seems to exist in the present case also. The other possibilities to explain the change associated with seismic activity could be dilatancy and dilatancy recovery[38]. If dilatancy in fault zone is manifested as opening of multiple penny-shaped vertical cracks, it produces a change in the gravity field which is poorly dependent on change in elevation. This phenomenon can occur in two environments, one in a horizontal shear stress regime leading to vertical strike slip faulting and the other in a tensional stress regime causing normal faulting[39]. As most of the minor and major events in this region have strike slip faulting with small normal component, a decrease in gravity field can be attributed to dilatation which is manifested in the form of opening of cracks. The increase can be attributed to dilatancy recovery. For a better understanding of the gravity changes in seismically active region, and its application as precursor studies, much more gravity, water level and geodetic observations from different seismically active regions are required.

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