CHAPTER IV

RESULTS AND DISCUSSION

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IV -1.1 Chicken Electroretinogram :-

The electrical response of the eye to illumination, the electroretinogram (ERG), has long been recognized as a compound potential resulting from the summation of currents contributed to extracellular spaces by multiple cellular elements. It is recorded between the front and back of the eye and consists of an initial corneal negativity, the a-wave, followed by a positive deflection of larger amplitude and slower time course, the b-wave, which is followed by a much longer positive potential, the c-wave. There is also an off response, the d-wave (Fig. 4.1).

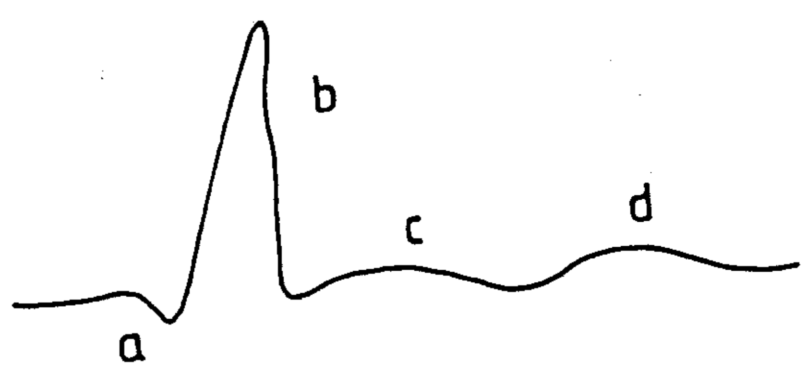

Fig (4.1): An ideal ERG wave.

Many factors affect the form and the components of the ERG such as the state of adaptation of the eye, age of the chicken, and its temperature.

Most studies on visual adaptation and age have been focused upon the changes in responses sensitivity (threshold) as a result of changes in background illuminance **(Kleinschmidt and Dowling, 1975; Green et al., 1975)** and age **(Fulton and Granes, 1978)**. So that in the present work the chicken age and the state of adaptation of the eye were taken into consideration during the ERG recording.

The retina is a neural network which is very sensitive to temperature. All elements comprising the retinal network, the retinal neurons and glial cells, and their functional interactions via synapses, are differentially sensitive to the effects of variations in temperature **(Negishi and Svaetichin, 1966, Schellart et al., 1974, Lamb, 1984; Armington and Adolph, 1984)**. These effects are therefore, reflected in the field potential corresponding to intraretinal pathway activity such as the ERG.

In this part of work, we shall study the ERG waves generated in both light and dark adapted chicken eyes and their changes due to temperature variations.

The ERG waves, as stated before, were recorded by the use of two disc Ag-Agcl electrodes, one being placed on the cornea and the other was inserted at the back of the eye. These electrodes were then connected to an oscillograph (Bioscience MD2). The optical stimulation of the eye was applied by the use of light flasher of intensity 4 lux. Typical records of the chicken ERG waves are shown in figure (4.2) for both light and dark adapted eyes at room temperature (23-25°C).

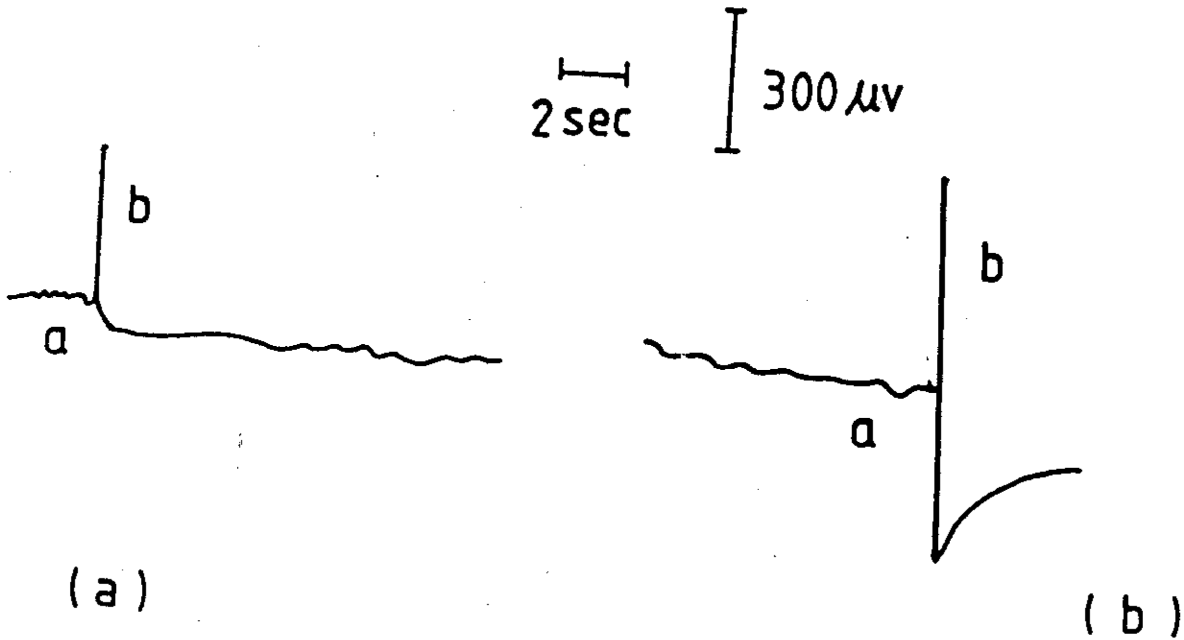

Fig (4.2) : Typical records of the chicken ERG waves of light (a) and dark (b) adapted eyes.

It is clear from fig(4.2)that the ERG waves in both cases, consist only of the a and b-waves while the c-and d are absent. This may be due to the used short flashes (<1sec) at which the c-wave does not appear and never outlasts light offset **(Wioland and Banaventures, 1984)**. There are several possible reasons for the disappearance of the c-wave some of these are :

1) The c-wave is known to be highly vulnerable whenever temperature or oxygen supply deviate from optimal conditions.
2) It may also be that morphological or functional differences among species provide more or less favourable conditions for K^+ mediated interaction between photoreceptor and pigment epithelial cells which is the source of the c-wave.
3) The overlapping of ERG components may obscure a small c-wave **(Matsura et al., 1978)**.

Therefore, in this work, we shall concentrate our attention on the recorded a & b-waves of the chicken ERG and their variations due to the effect of temperature, conditions of adaptation and argon laser exposures.

IV-1.2.Effect of the temperature variations on the chicken ERG

According to the results obtained in the present work the values of the ERG b-wave amplitude were changed with the age of the chicken. This was also reported by **Fulton and Granes, 1978.** It reached an approximately a constant value in case of chicken age between 2-3 weeks. So, in all of our experiments, the age was fixed at this range. Hence, in the present work, chicken of age 2-3 weak, were chosen. After bird decapitation, the eye was enuculated and placed in the recording chamber through which Ringer's solution with different temperatures, from 10-30°C, in

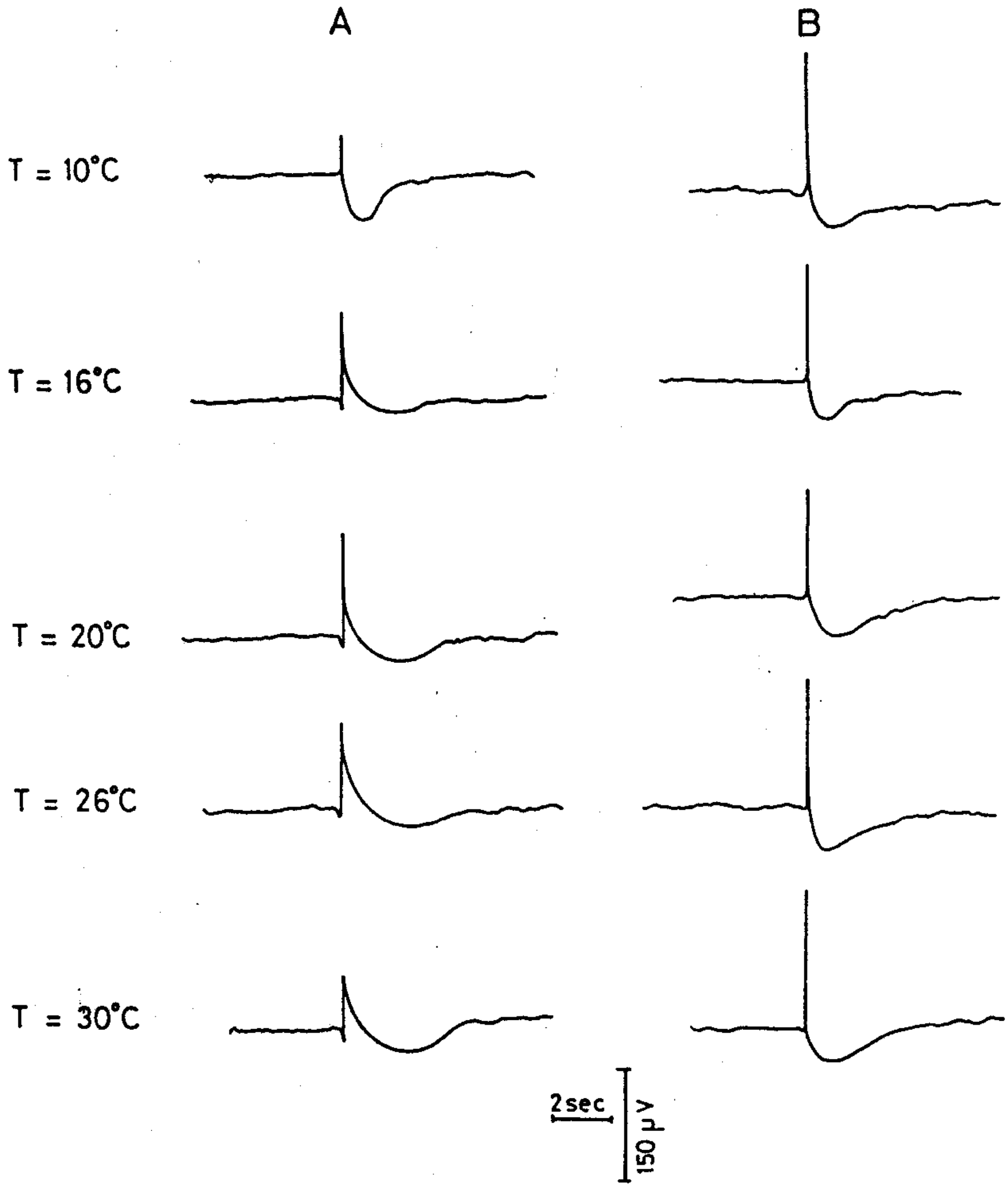

Fig.4.3 Typical records of ERG recorded from light (A) and dark (B) adapted eyes at temperatures from 10°C up to 30°C.

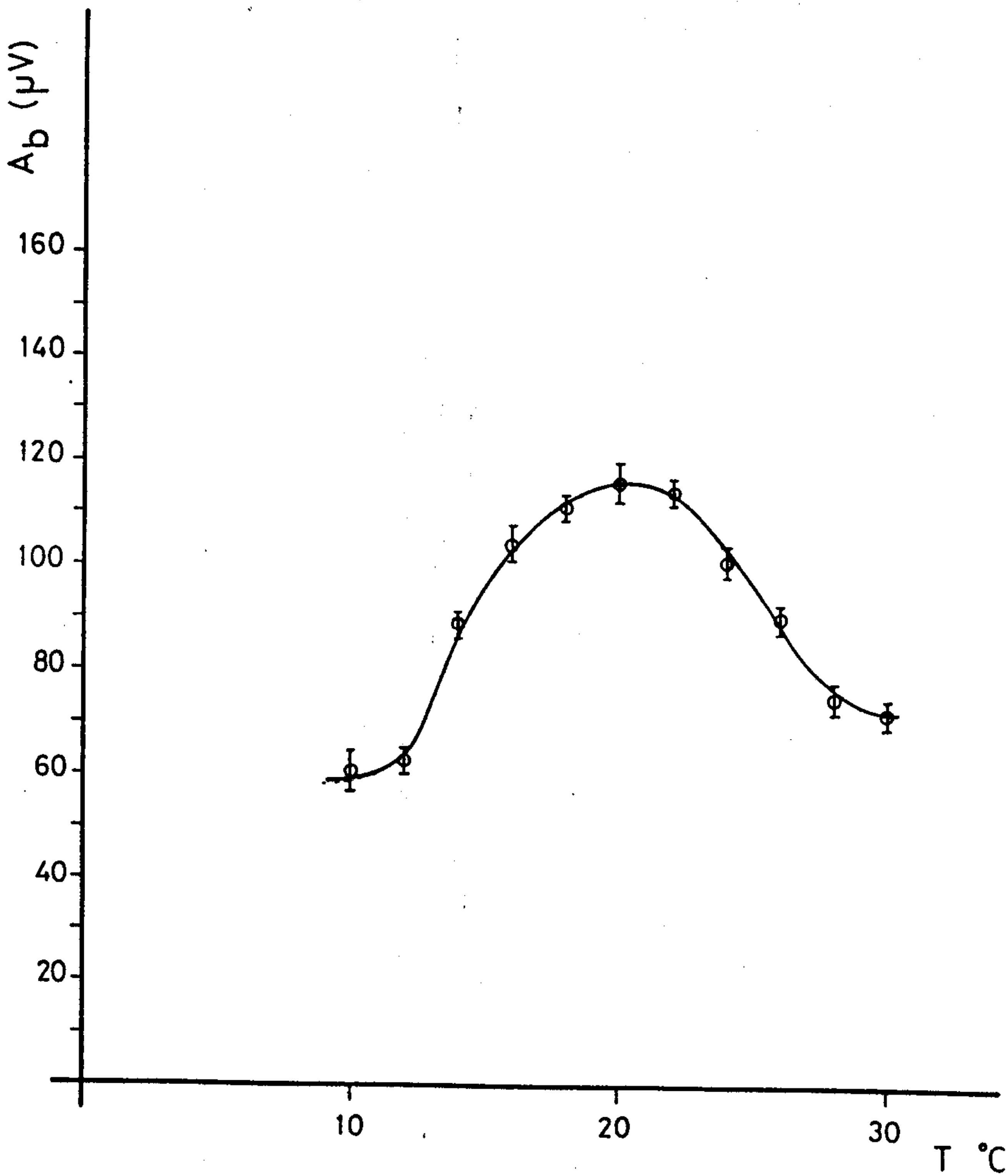

Fig.4.4 The relation between the temperatures °C and the b-wave amplitude (A_b) of light adapted chicken eye.

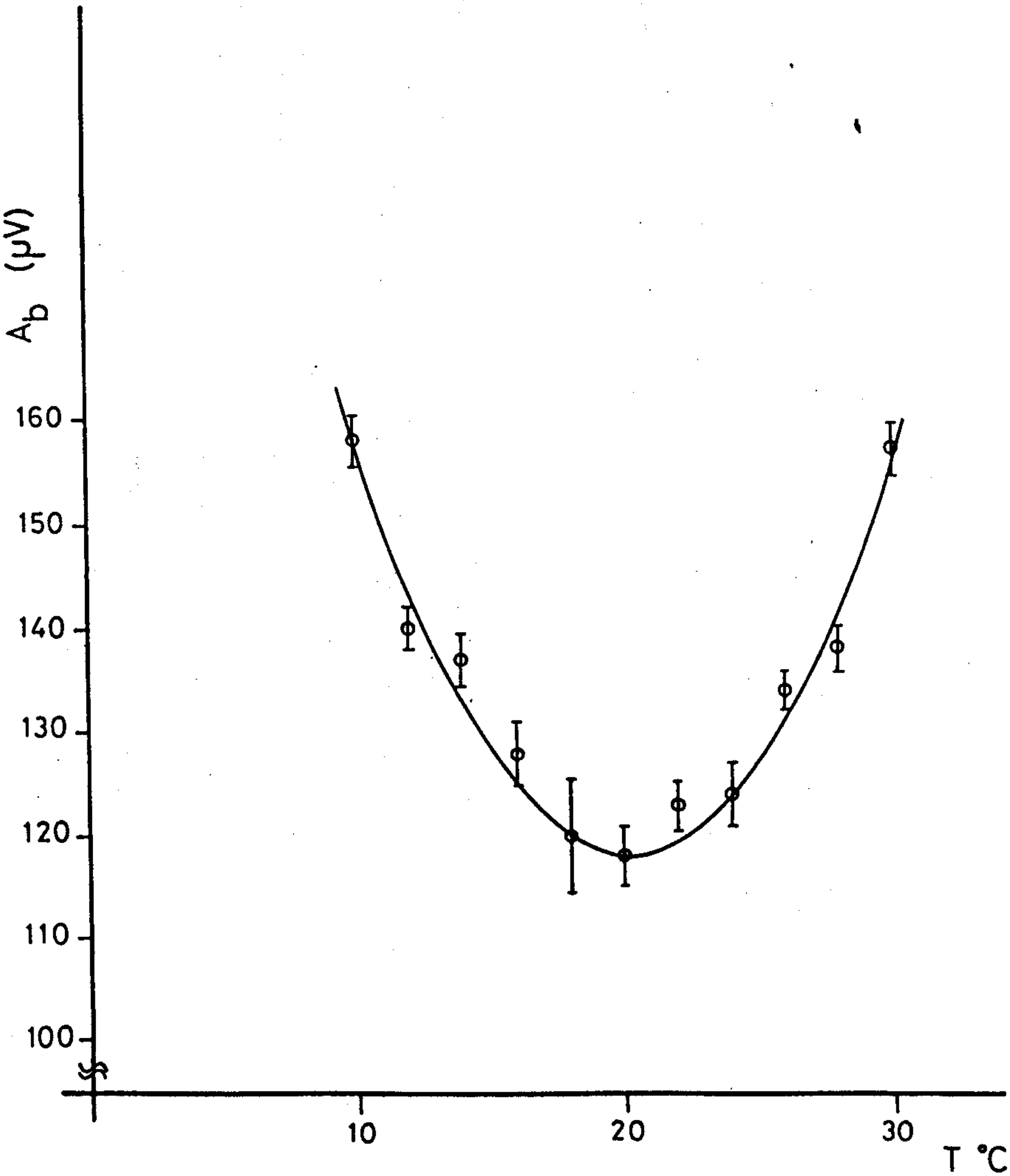

Fig. 4.5 The relation between the temperatures °C and the b-wave amplitude (A_b) of dark adapted chicken eye.

steps of 2°C was circulated. The temperature was maintained at each desired degree for a period of 10 minutes in order to record the ERG waves several times through this interval. The experiments were repeated on 8 eyes in both light and dark adaptation and the ERG was recorded at each temperature.

Typical examples of ERG records from light and dark adapted eyes at a temperature variations, in the range 10-30°C, are shown in figure (4.3). It shows that the b-wave of the recorded ERG, in both cases of light and dark adaptation, is very sensitive to temperature variations more than the a-wave.

The data of the variation of b-wave amplitudes, with temperature are tabulated in table (4.1) and represented in figures (4.4) and (4.5). IN case of light adapted eyes (figure 4.4), the amplitude of the b-wave (A_b) showed an increase at the intermediate values of temperature (18-22°C) but decreased at both lower (10-18 °C) and higher (22-30 °C) values. Other while, in case of dark adapted eyes (Fig. 4.5) the amplitude-temperature relationship shows a quit opposite manner in behaviour to that of light adapted one. They showed a decrease at the intermediate values of (18-22°C) and an increase in the lower and higher values.

In addition to temperature sensitivity changes of the ERG b-wave, also The wave-form and its variations with temperature is altered during dark and light adaptation.

The photopic curve (of light adapted eyes) of figure (4.4) behave in quite opposite manner than the scotopic curve (of dark adapted eyes) of figure (4.5).These changes of the b-wave during light and dark adaptation may be attributable to changes in response waveforms of individual cells in the visual system **(Schickman, 1981, Ripps and Weale, 1976)**. This finding is in complete agreement with **Samuel et al., 1987**, who attributed that the changes of the response waveform of retinal horizontal cells during dark and light adapted eyes are due to the sensitivity changes. They also added that a hyperpolarizing response of the horizontal cells was obtained for light adapted eyes while depolarizing response was obtained for dark adapted eyes and this response is independent of the resting potential during dark and light adaptation. This suggests that a pre-synaptic element may be responsible for mediating such responses.

Photoreceptor cells are also varied in response during light and dark adaptation **(Samuel et al., 1987)**. The rod was never responsive to light flashes after bleaching whereas the cone gave a smaller response at both the onset and offset of the test flash.

Based on the above discussion, it is obvious that, most of the retinal cells behaves with quite opposite manner in case of light and dark adapted eyes. Such behaviour is reflected on the ERG waves recorded in both cases of adaptation and hence in good agreement with our obtained results.

The ERG a-wave, which reflects the receptor activity is less temperature sensitive than the b-wave, which reflects the muller cell activity and, indirectly, the depolarization of the bipolar cells **(Dick and Miller, 1978)**. These effects are similar to those obtained in case of the carp retina **(Armington et al., 1984)**.

The experimental findings concerning the temperature sensitivity of the chicken retina as reflected in the ERG properties (especially in the b-wave) and their implications about the functional stability of various intraretinal pathways under varying temperature conditions are very clear. So, the great thermal effects of some visual stimuli (such as in diathermy) which is used to treat retinal diseases must be taken into account in order to prevent severe defects on the eye. There for, it is of important to maintain the temperature at a suitable value during retinal experiments or treatment.

Determination of this suitable temperature, during the laser application on the eye, is the aim of the next part of this work.

IV- 1.3 ·**Effect of laser on the chicken ERG response :-**

Since many years ophthalmologists have used light in the treatment of eye diseases. The xenon are lamp, which used to be employed for retinal photocoagulation, has been replaced by laser. It is mainly used in the treatment of peripheral retinal vascular abnormalities (especially diabetic retinopathy) and peripheral retinal detachment and retinal tears.

Treatment, is usually performed with an argon laser which emits two lines at 488 and 515 nm. It is usually selected, a suitable wavelength, power and duration of exposure, according to the desired effect.

The ERG is the most important indicator for the validity of the retina. It is a direct reflection of any damage/repair, which may occur, during laser treatments.

So, in this work, we shall study the ERG waves generated in both light and dark adapted chicken eyes (2-3 weeks age) and its variations when the eyes have been exposed to argon laser beam of the same intensity about $1mw/cm^2$ and duration, 0.12 and 0.25 sec, as that used in medical laser treatments. It would be important to carry out the ERG records at different temperatures (from 10°-30°C) in order to find the best suitable conditions to develop the critical balance of damage/repair rates during laser treatments.

a- Effect of laser on the ERG of light adapted eyes :

The ERG records were carried out before, immediately and after 1,2,3,4 and 5 days of laser exposure of intensity $1mw/cm^2$, durations of 0.12 & 0.25 sec and at different temperatures from 10-30°C in steps of 2°C. Examples of the ERG records at a temperature of 20°C are shown in Fig. (4.6). The data of the b-wave amplitudes at different temperatures (10-30°C) and laser durations 0.12 & 0.25 sec are tabulated in table (4.2) and represented in figures (4.7) and (4.8) respectively. They indicated that the b-wave amplitude (A_b)-temperature relationships show the same behaviour in all the days before and after laser exposure.Also,the A_b takes maximum values at a temperature in the range of 15-20°C. On the other hand, A_b shows fluctuated decrease in their values during the first three days after laser exposure and then they may approach certain recovery in the fourth or fifth day.

Regarding the two figures (4.7) & (4.8), it is very difficult to differentiate or compare between the laser effects in both duration of 0.12 & 0.25 sec since no significant difference is found between them. So, in order to overcome this difficulty, the percentage relative changes in the b-wave amplitudes ($\frac{\Delta A_b}{A_b}$%) with respect to the normal values (A_b) are plotted with time, after laser exposure and at temperatures 10,20 & 30°C for both laser durations 0.12 & 0.25 sec, as shown in figures (4.9) & (4.10). They indicated that :

The effect of temperature variations is not significant in case of duration 0.12 sec whilst it is more pronounced at duration 0.25 sec. The changes of $\frac{\Delta A_b}{A_b}$ % are more pronounced at a temperature of 10°C followed by 20°C & 30°C respectivelv. Moreover, at laser duration of 0.12 sec the value of($\frac{\Delta A_b}{A_b}$%)approached a certain recovery

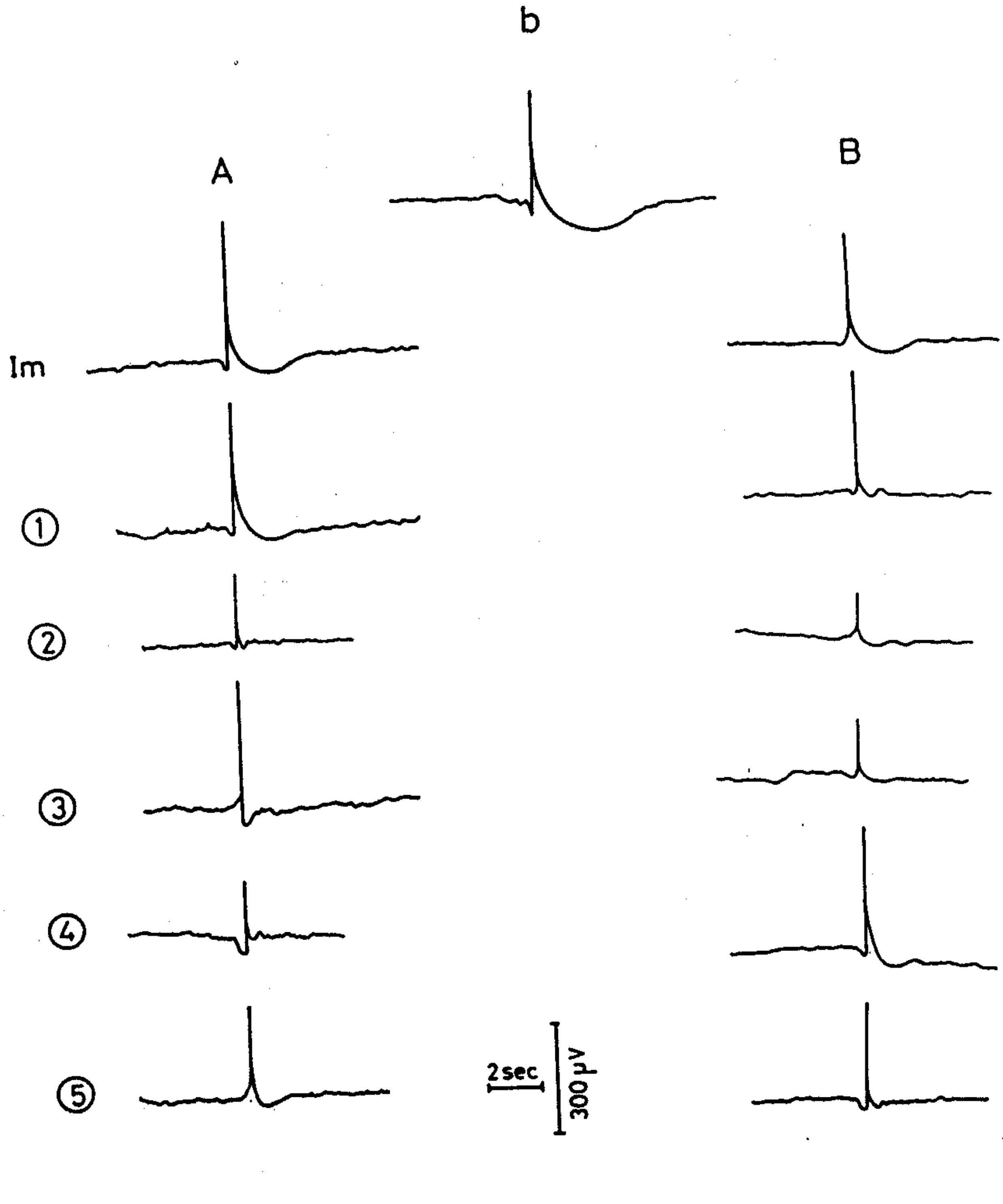

Fig. 4.6 ERG waves recorded before (b), immediately (Im) and after (1 - 5) days of laser exposure of durations 0.12 (A) and 0.25 sec (B) to light adapted eyes at a temperature of 20°C.

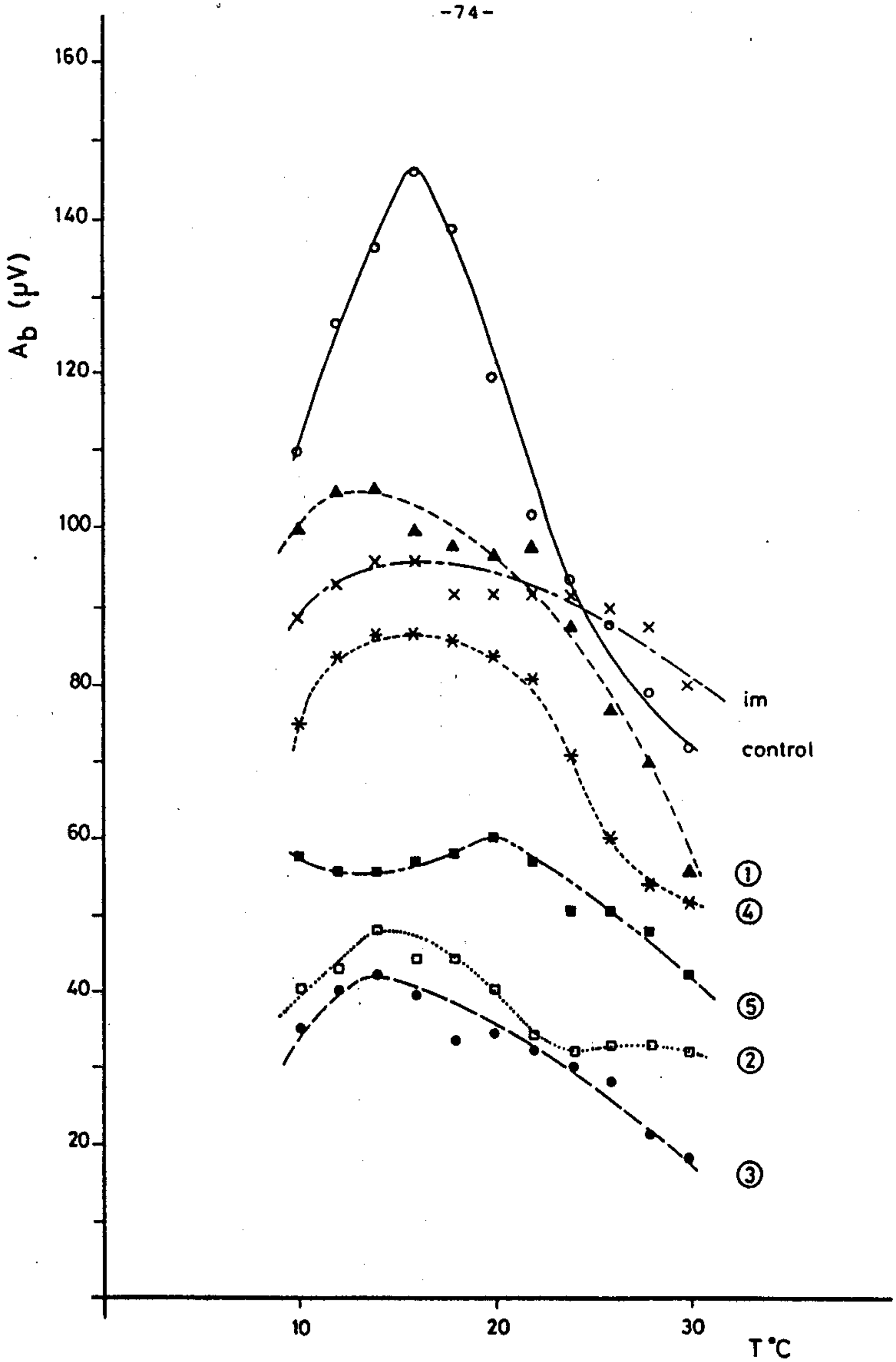

Fig.4.7 Relation between b-wave amplitude (A_b) and temperature before (control), immediately (im) and after 1, 2, 3, 4 and 5 days of light adapted eye laser exposure of duration 0.12 sec.

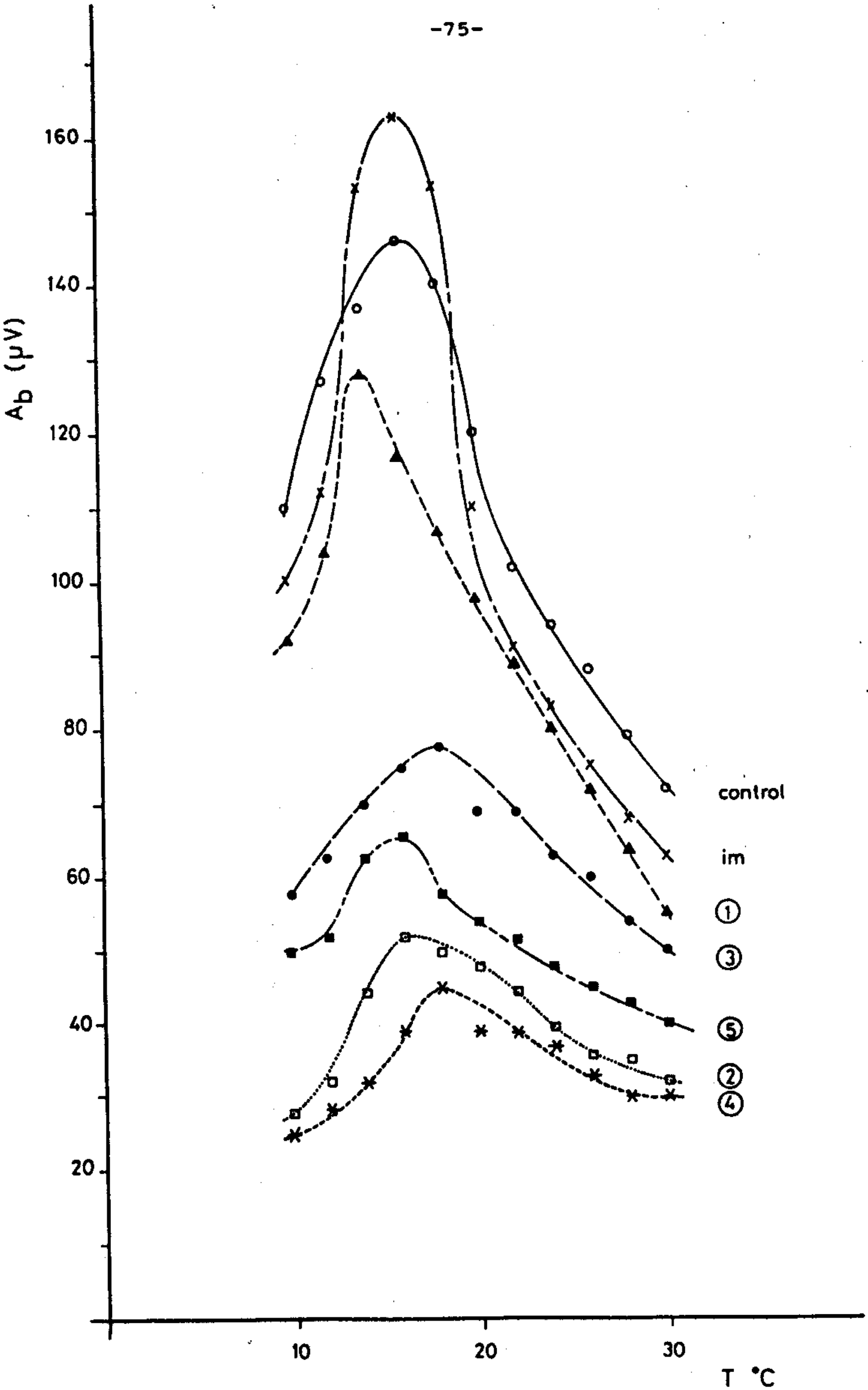

Fig.4.8 Relation between b-wave amplitude (A_b) and temperature before (control), immediately (im) and after 1, 2, 3, 4 and 5 days of light adapted eye laser exposure of duration 0.25 sec.

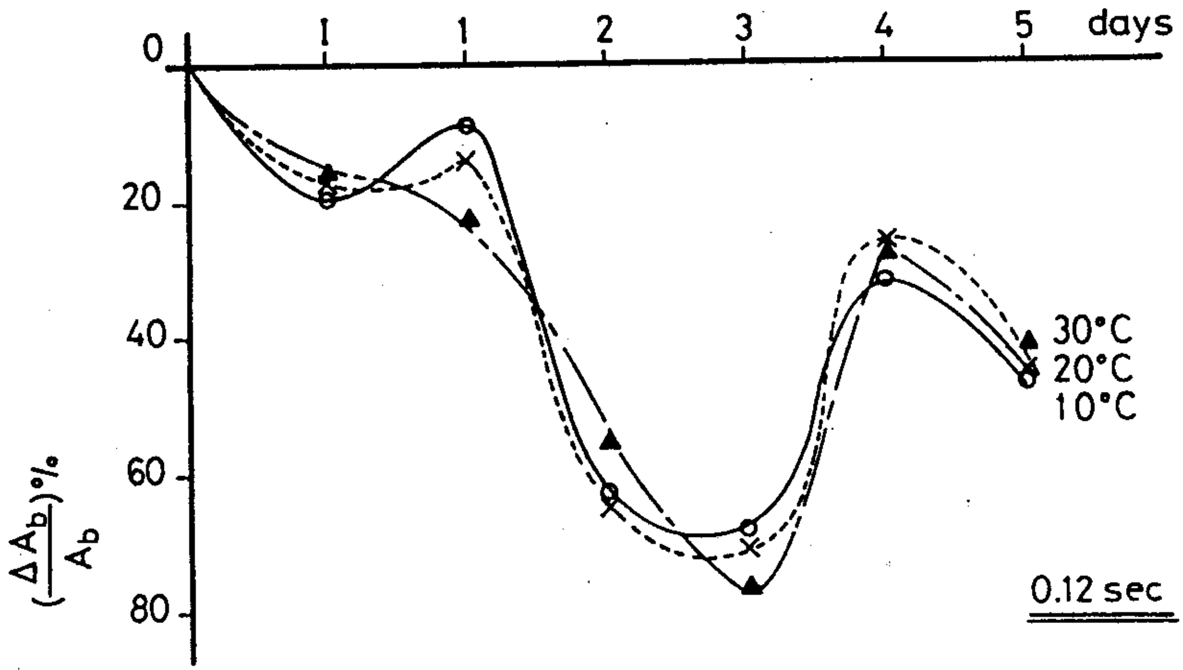

Fig. 4.9 The percentage relative changes in the b-wave amplitudes $(\Delta A_b/A_b)\%$ after immediate (I) and 1, 2, 3, 4 and 5 days of laser exposure of duration 0.12 sec and constant temperatures 10, 20 and 30°C. Light adapted

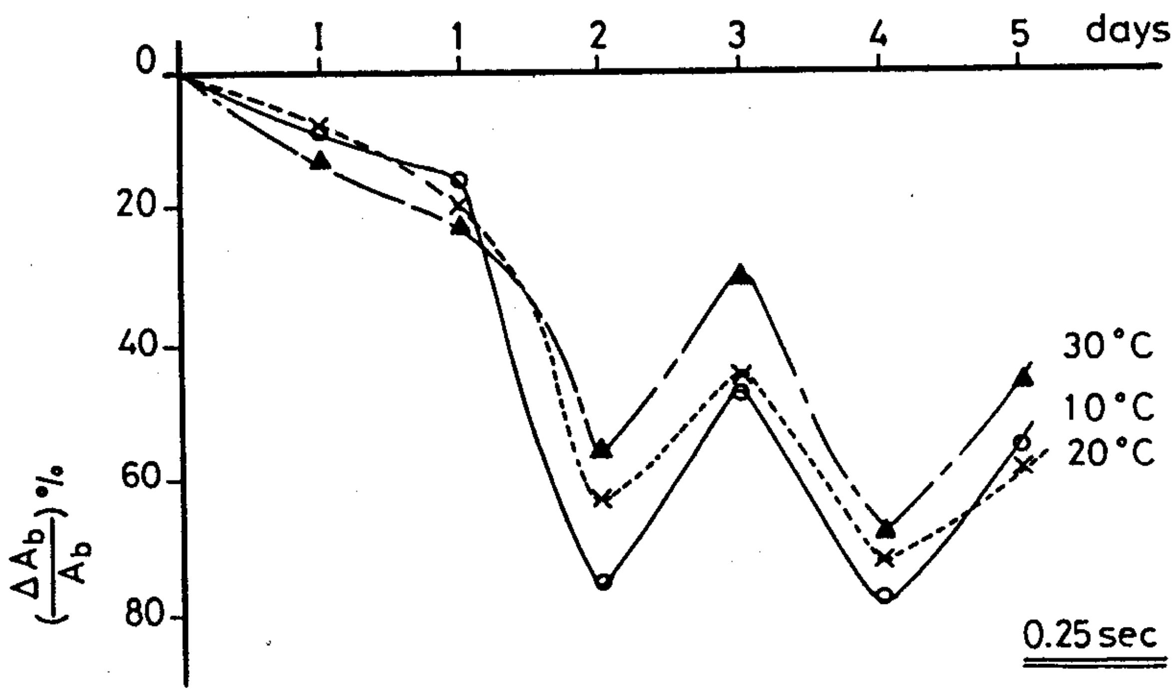

Fig. 4.10 The percentage relative changes in the b-wave amplitudes $(\Delta A_b/A_b)\%$ after immediate (I) and 1, 2, 3, 4 and 5 days of laser exposure of duration 0.25 sec and constant temperatures 10, 20 and 30°C. Light adapted

T °C	Control	Mean b-wave amplitude (MV) ± SD											
		0.12 sec						0.25 sec					
		im.	1	2	3	4	5	im.	1	2	3	4	5
10	110± 3.2	89± 1.3	100± 3.1	40± .1	35± .4	75± 1.2	58± 1.0	100± .7	92± .8	28± .2	58± .12	25± .2	50± .2
12	127± 1.4	93± 1.8	105± .7	43±.56	40± 1.5	84± .4	56± .6	112± .9	104± .9	32± .4	63± .9	28± .3	52± .3
14	137± 2.8	96± 1.5	105± .5	84± .4	42± 1.1	87± .36	56± .5	153± .3	128± 1	44± .3	70±1.1	32± .5	63± .4
16	147± 4.5	96± 1.5	100± .35	44± 1.1	39± 1.0	87± 2.5	57± 1.1	163± .4	117± 0.5	52± .4	75±1.4	39± .4	66± .6
18	140± 5.3	92± 92	98± 2.9	44± .37	33± 1.4	86± 1.7	58± 1.5	153± .8	107± .5	50± .5	78± .5	45± .5	58± .6
20	120± 5	92± 2.8	97± .6	40± 1.5	34± .2	84± .48	60± .9	110±1.0	98± .3	48± .4	69± .4	39± .6	54± .7
22	102± 4	92± 2.8	98± .72	34± .5	32± .5	81± .9	57± .2	91± .3	89± .5	44± .3	69± .5	39± .7	52± .4
24	94± 2.3	92± 3.2	88± .31	32± .4	30± .6	71± .95	51± .5	83± .9	80± .6	39±.23	63± .2	37± .8	48± .4
26	88± 1	90± .5	77± .6	33± .6	28± 2.7	60± .83	51± .5	75± .4	72± .4	36± .1	60± .7	33± .4	45± .3
28	79± 2.9	88± .4	70± .2	33± .5	21± .3	54± .75	48± .8	68± .5	64± .2	35± .3	54± .4	30± .5	43± .3
30	72± 2.9	80± .3	65± .32	32± 0.2	17± .42	52± 1.1	42± 1	63± .2	56± 1.8	32± .4	50± .5	30± .5	40± .3

Table (4.2): Data of temperature and the corresponding b-wave amplitudes before (control), immediately (1m) and after 1,2,3 4, + 5 days of light adapted eyes laser exposure of duration 0.12 + 0.25 sec.

b- Effect of laser on the ERG of dark adapted eyes :

The experiments of section (a) were repeated in case of dark adapted eyes. Examples of records, at a temperature of 20°C are shown in fig. (4.11). The obtained results, that is the variation of A_b waves with respect to temperature and time after laser exposures of durations (0.12, 0.25 sec) are represented in Fig (4.12) & (4.13) and tabulated in tables (4.3 & 4.4).

The b-wave amplitudes showed a fluctuated decrease, from their normal values, in all the days after laser exposure of both durations. Their minimum values occurred at a temperature of 18-20°C.

Since the differences between the effects of both laser duration are not noticeable, then it could be more illustrative as represented in figures (4.14) and (4.15)

which show the percentage relative changes of the b-wave amplitudes $(\frac{\Delta A_b}{A_b}\%)$ with respect to the normal value A_b with with time (in days) after laser exposure at a temperature of 10,20 & 30°C. They indicated that there is no complete recovery in the fifth day following exposure while it seems to approach recovery in the fourth and fifth days for laser duration of 0.12 and 0.25 sec respectively. This recovery is more pronounced in case of temperature 10°C than that in case of 20°C and 30°C.

The experimental findings concerning the laser sensitivity of the chicken retina as reflected in the ERG properties, especially in its b-wave and its implications

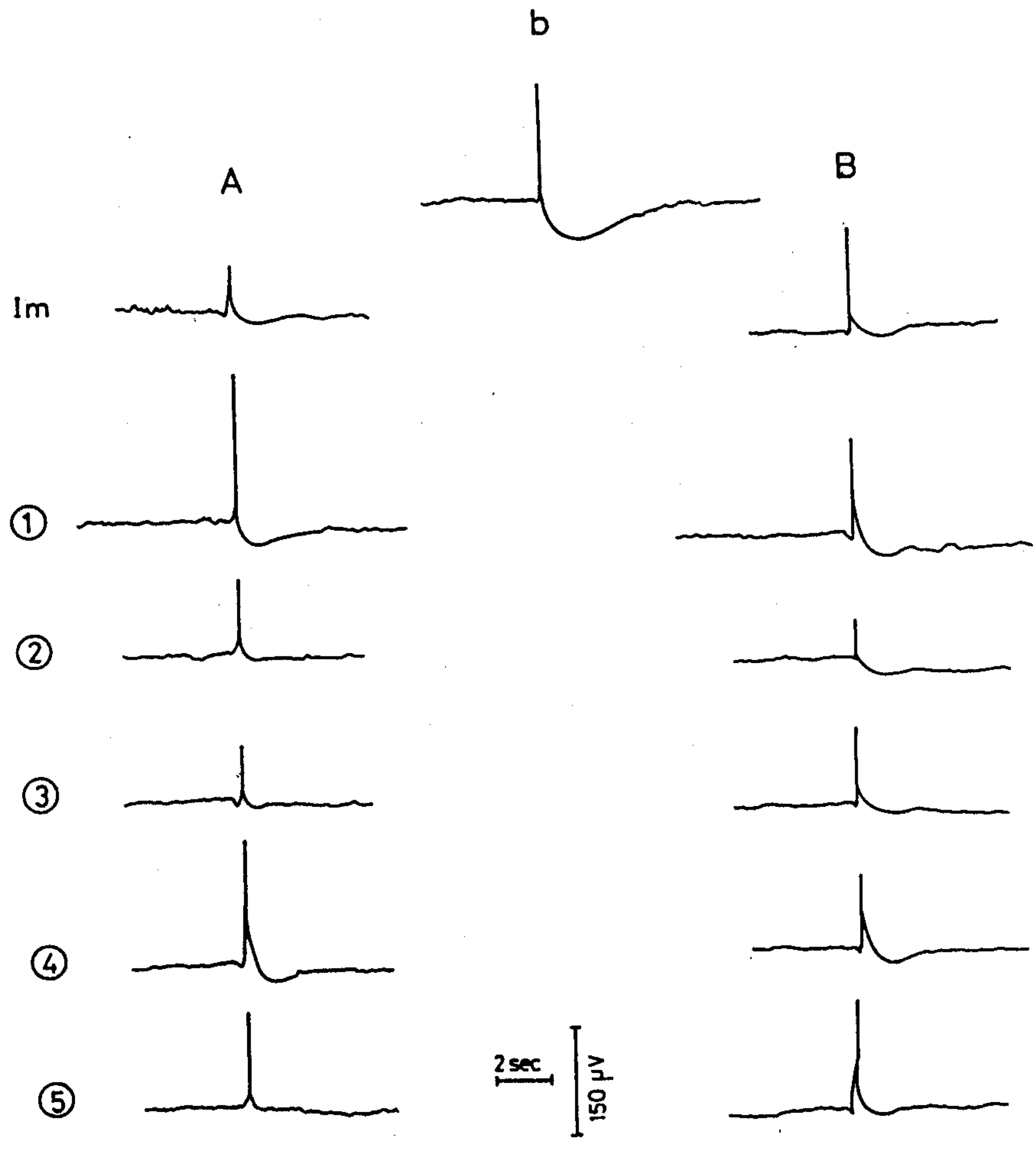

Fig.4.11 . ERG records before (b), immediately (Im) and after (1 - 5) days of laser exposure of durations 0.12 (A) and 0.25 sec (B) at a temperature of 20°C. (dark adapted eyes)

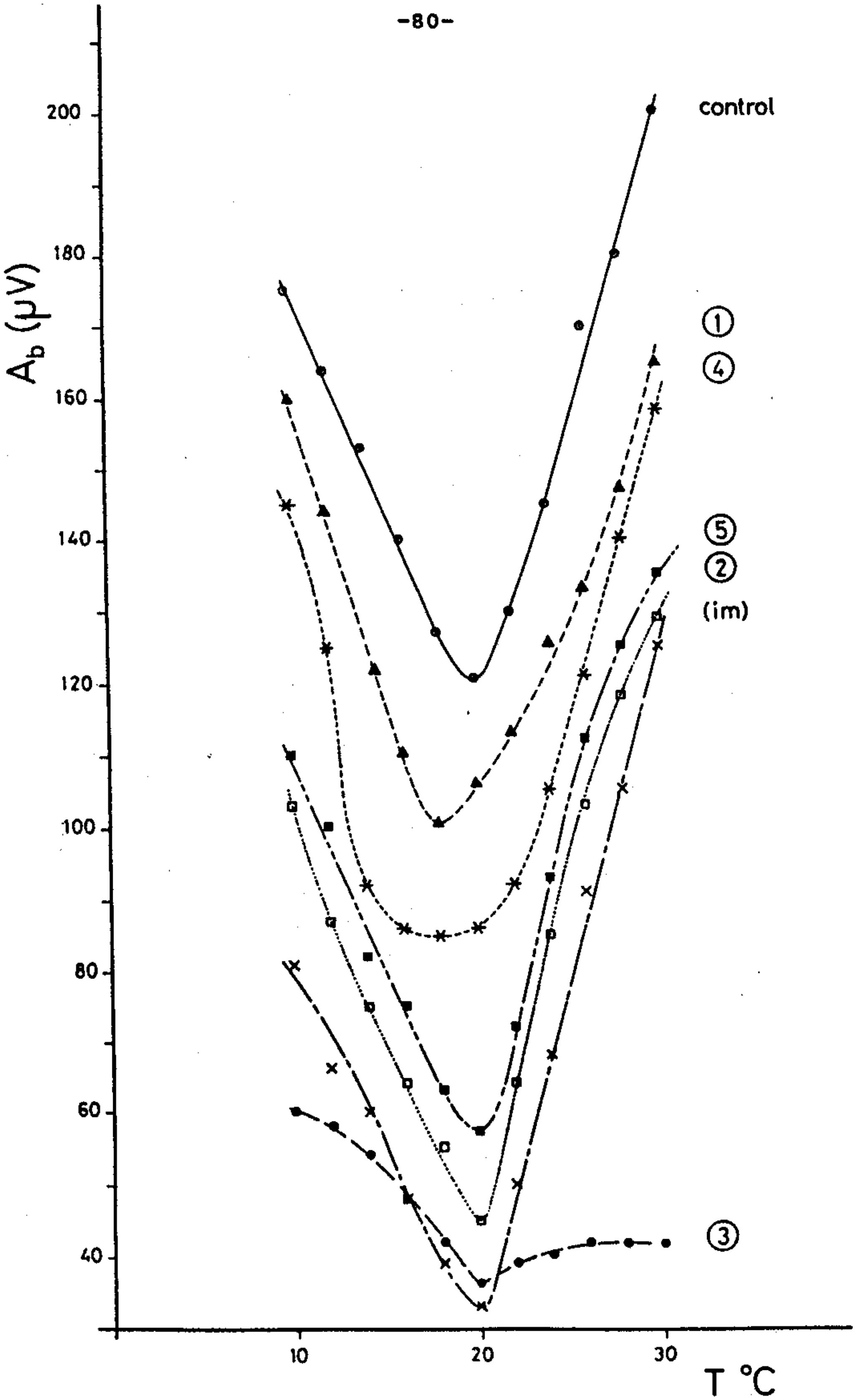

Fig.4.12 Relation between b-wave amplitude (A_b) and temperature before (control), immediately (im) and after 1, 2, 3, 4 and 5 days of laser exposure of duration 0.12 sec. (dark adapted)

indicated that the b-wave amplitudes showed pronounced variations when we expose the chicken eyes to laser of duration 0.12 & 0.25 sec in both cases of light and dark adaptation. The reason for these variation may be due to that the used laser of intensity (1mw) and duration (0.12 & 0.25 sec), which were the same as those used in laser treatments, produce their little damage primarily in the outer layers of the retina, without touching the ganglion cells or nerve fiber layer **(Powell et al., 1971, Apple et al., 1973).**

These effects appeared as a reduction in the b-wave amplitudes and this reduction decreased and b-wave approach complete recovery in the fourth or fifth day after laser exposure depending on the exposure dose (D=It where I is the laser intensity and T its duration).

The state of eye adaptation is an important factor during laser treatment and it is clear that the results, in case of dark adapted eye, were more representative and regular than that of light adapted one because of the high sensitivity of the eye in the first case.

Also, the temperature play an important role during laser exposure, and as we decreased it (e.g. fig 4.9 & 4.10) more approach to recovery was occurred since the cooling effect may oppose the laser heat effect during treatments.

Accordingly, we may conclude that, patients who have to undergo retinal laser therapy should undergo careful and frequent ERG examinations before and after treatments and it is useful in the treatment to decrease the laser dose and the temperature and to perform it in case of dark adaptation in order to decrease the damage/repair or risk-benefit ratio which is extremely small by comparison with many other medical and surgical therapies.

IV.2- Variation of dielectric constant of chicken eye subjected to laser beam at different temperatures.

Physical stimuli affects the function, the structures and the bioelectrical properties of the eye. Among those are the temperature and the laser beam.

Argon laser is now used as a tool in both diagnosis and operation in ophthalmology. Then it is very important to study its effect on the structure and function of the eye immediately and after a certain time of exposure. Hence, one of the aims of the present work is to define the most suitable conditions of laser application on the eye producing the minimum damage. In this part of the present work we intended to use the argon laser with the same intensity and duration as that used in the retinal operation.

In addition, the structure and functional states of the eye exposed to laser, are studied by the use of the dielectric methods which showed a great sensitivity for such changes. Also a great attention had been paid to study the laser late effects in order to test the recovery processes in the retina which is of great interest.

The experiments were carried out on the whole eye of chicken placed in the dielectric cell and hence the dielectric measurement were performed using the impedance bridge operated in the frequency range 0.5-50 KHz. The control of the sample temperature was achieved by passing water through the water jacket coaxially surrounding the sample cell. Which has two disc Ag Agcl electrodes each having an area of about 0.785 Cm^2 with an interelectrode distance of 0.7 Cm.

The relative permittivity ϵ', was calculated from the following equations by using impedance values Z_I together with the phase angle Φ.

$$C = \frac{1}{JW|Z| \sin \phi} \quad \text{and} \quad C = \epsilon' \epsilon_o A/d \quad \text{---------(4.1)}$$

where C is the calculated capacity in fared.

ϵ_o is the permittivity of free space.

A is the area of the electrode in m^2.

and d is the separation distance in meter between the two electrodes.

The dieleotric loss, ϵ'', can be calculated from the

$$\text{equation : } \epsilon'' = \epsilon' \tan (\delta) \quad \text{----------------(4.2)}$$

where (δ) is the loss angle given by

$$\tan (\delta) = \tan (\phi - 90) \quad \text{------------(4.3)}$$

The variations of the dielectric parameters in case of light adapted eyes showed great variations rather than that of dark adaptation.

So that in this work we shall demonstrate the results obtained in case of the dark adapted eyes and also the main differences between them.

As a matter of fact, any biological tissue exposed to a laser beam of a certain intensity its temperature will be increased. So it is of great important, first, to study the effect of temperature variation on the eye function before applying of the laser beam.

Figure (4.16) shows three semicircles which demonstrate the relation between the real impedance Z_R and the imaginary one Z_I of dark adapted chicken eye placed at different temperatures of 10.20 & 30°C. Each point has a standard deviation less than 0.07 and represents the mean of five experiments carried out on different dark adapted chicken of approximately the same age (2-3 weeks). As clear from fig (4.16), the centre of the semicircles is not located at the x-axis but deviated below the centre and hence making the angle θ as previously mentioned before chapter (III).

This angle is found to vary with the temperature. Their values were 82°, 89°, & 88° at temperatures 10,20 & 30°C respectively.

It should be noted that the intercept of the semicircle with the x-axis represents the value of the resistance of the chicken eye at each temperature. This value, at 20°C was more than those at 10 and 30°C.

In the following parts, the effects of the argon laser beam, of fixed intensity and different durations 0.12 and 0.25 sec, on the functions and properties of dark and light adapted chicken eyes placed at different temperatures, are studies.

Fig. (4.16) Shows a set of semicircles which demonstrate the relation between the real impedance Z_R and the

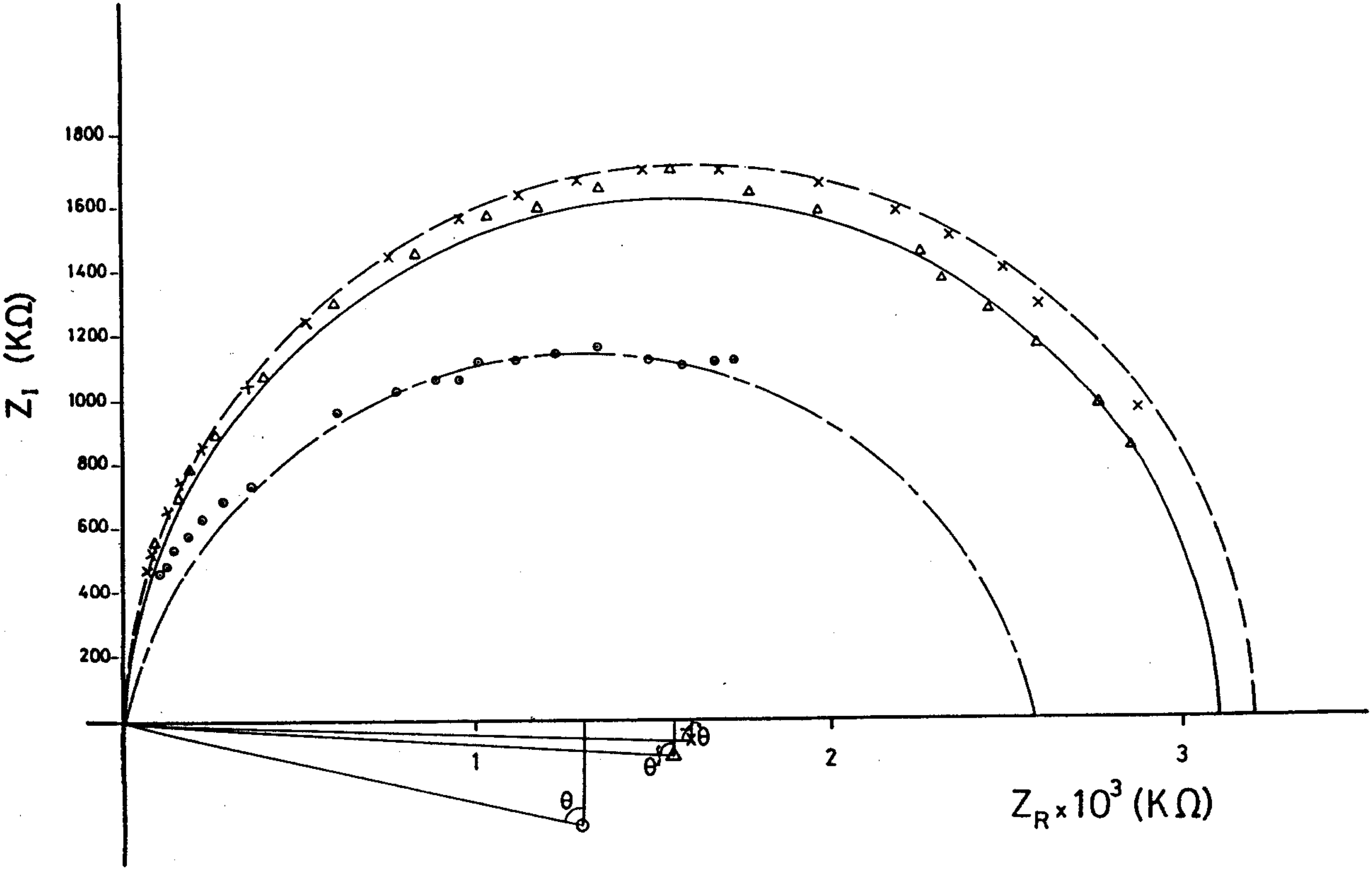

Fig. 4.16 The relation between the real (Z_R) and imaginary (Z_I) parts of dark adapted chicken eye impedance at normal conditions and different temperatures (⊙ — 10°C, x — 20°C and Δ — 30°C).

imaginary Z_I of dark adapted chicken eye exposed to laser beam of intensity 1 mw/cm2 and duration 0.12 sec at a fixed temperatures of 10, 20 and 30 °C respectively. Each point has a standard deviation of about 0.07 and represents the mean of five experiments carried out on different dark adapted chicken of approximately the same age. As shown from figure 4.17 (2 a, b & c).

Each set of semicircles represents the obtained results in case of unexposed chicken eye and those exposed immediately and after five recovery days at which the exposed bird was living in a dim light before decapitation. In this figure the angle θ previously described in chapter (III) is found to vary during the immediate and the five recovery days at each constant temperature (10, 20 & 30 °C). It decreases at the first recovery day and then increases toward the normal value and could be used as indication for the repairing processes. It should be noted that the intercept of the semicircle with the X-axis, representing the resistance of the chicken eye, is different for the immediate exposure and the recovery days Fig. 4.17 (a, b & c). It shows a great variations at the temperature of 20 °C.

The variation of angle θ due to changes in temperature may be due to the transient behaviours as a function of time, such as the change in the potential V after a sudden change of current as stated by **Weber (1954)**.

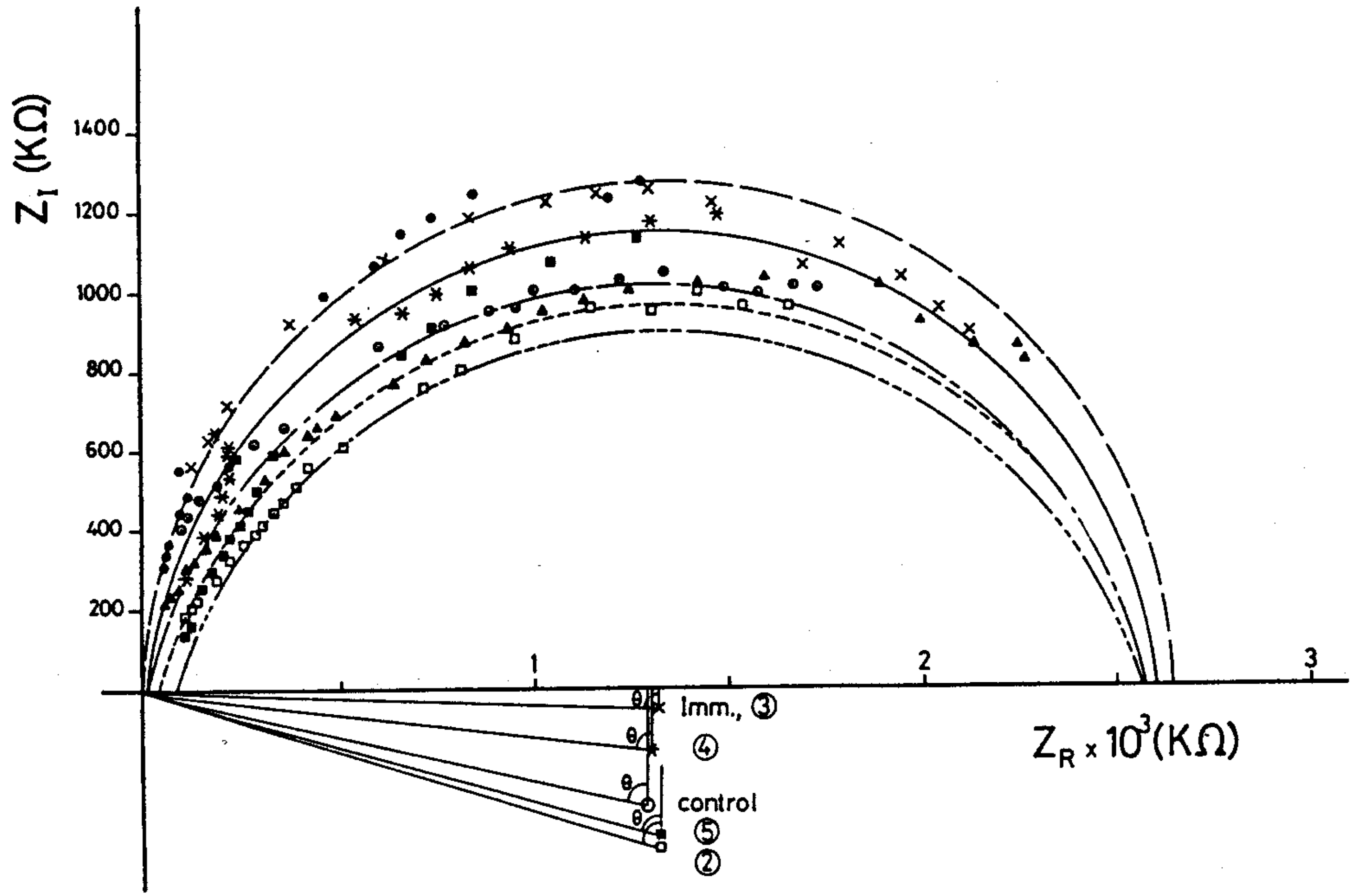

Fig. (4.17)a The relation between the real (Z_R) and imaginary (Z_I) parts of dark adapted chicken eye impedance at constant temperature 10°C exposed to laser beam of duration 0.12 sec immediately and for 5 recovery days (control ⊙, immediately x, 1st Δ, 2nd □, 3rd ●, 4th * and 5th □).

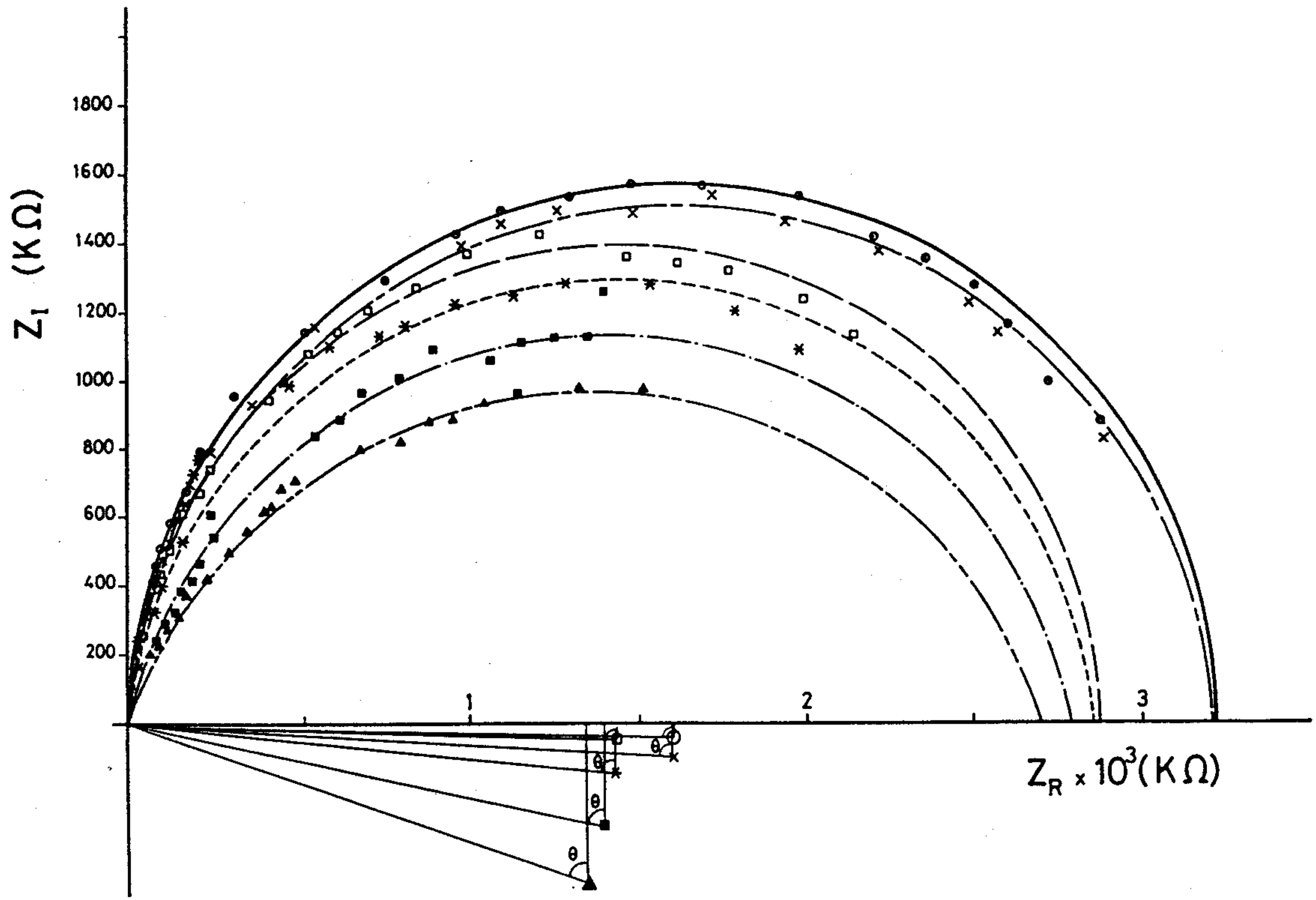

Fig. (4.17)b The relation between the real (Z_R) and imaginary (Z_I) parts of dark adapted chicken eye impedance at constant temperature 20°C exposed to laser beam of duration 0.12 sec immediately and for 5 recovery days (control ⊙, immediately x, 1st Δ, 2nd □, 3rd ●, 4th * and 5th □).

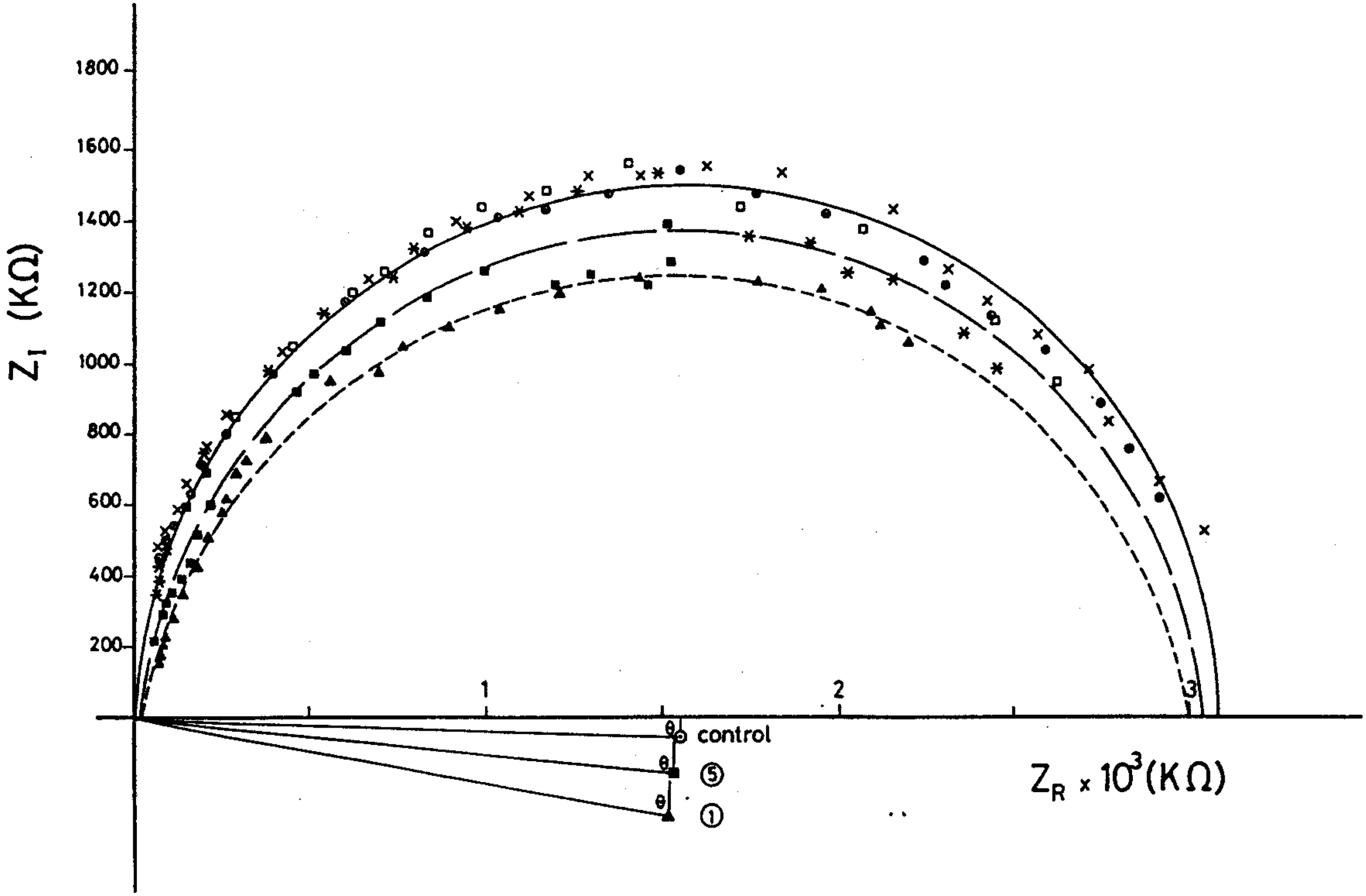

Fig. (4.17)c The relation between the real (Z_R) and imaginary (Z_I) parts of dark adapted chicken eye impedance at constant temperature 30°C exposed to laser beam of duration 0.12 sec immediately and for 5 recovery days (control⊙, immediately x, 1st Δ, 2nd □, 3rd ●, 4th * and 5th □).

From the values of the impedance and the phase angle obtained from the previous obtained results, demonstrated in Fig. 4.17 (a,b, & c) and Fig. 4.18 (a, b, & c) is constructed.It shows a set of curves demonstrate the relation between the dielectric loss ϵ'' and the logarithmic values of the frequency, in the range of 0.5 - 50 KHZ, for dark adapted chicken eye exposed to laser beam of intensity $1 mw/cm^2$ and duration 0.12 sec at constant temperatures 10, 20 and 30 °C respectively. Each set of curves represents the obtained results in case of three conditions, control, immediate and the fourth day of recovery. We chose these days because the repairing processes began to reach a certain rate in comparison with the immediate and first days of recovery. The results of Fig. 4.18 (a, b & c) showed that all the demonstrated curves have the same behaviour and the maximum value of dielectric loss ϵ'' occurs at frequency range from 900 - 2000 HZ, increases at the immediate exposure and then decreases in the fourth recovery day. The results also showed that the maximum dielectric loss varies with the temperature and after laser exposure. It reached a maximum value at 30 °C and immediately after laser exposure. The previous experiments were repeated on the dark adapted chicken eye when exposed to argon laser of the same intensity but with duration 0.25 sec. The experimental data are tabulated in table (4.5) together with that of 0.12 sec duration. As seen from the data of table (4.5) that there are no significant changes in the dielectric

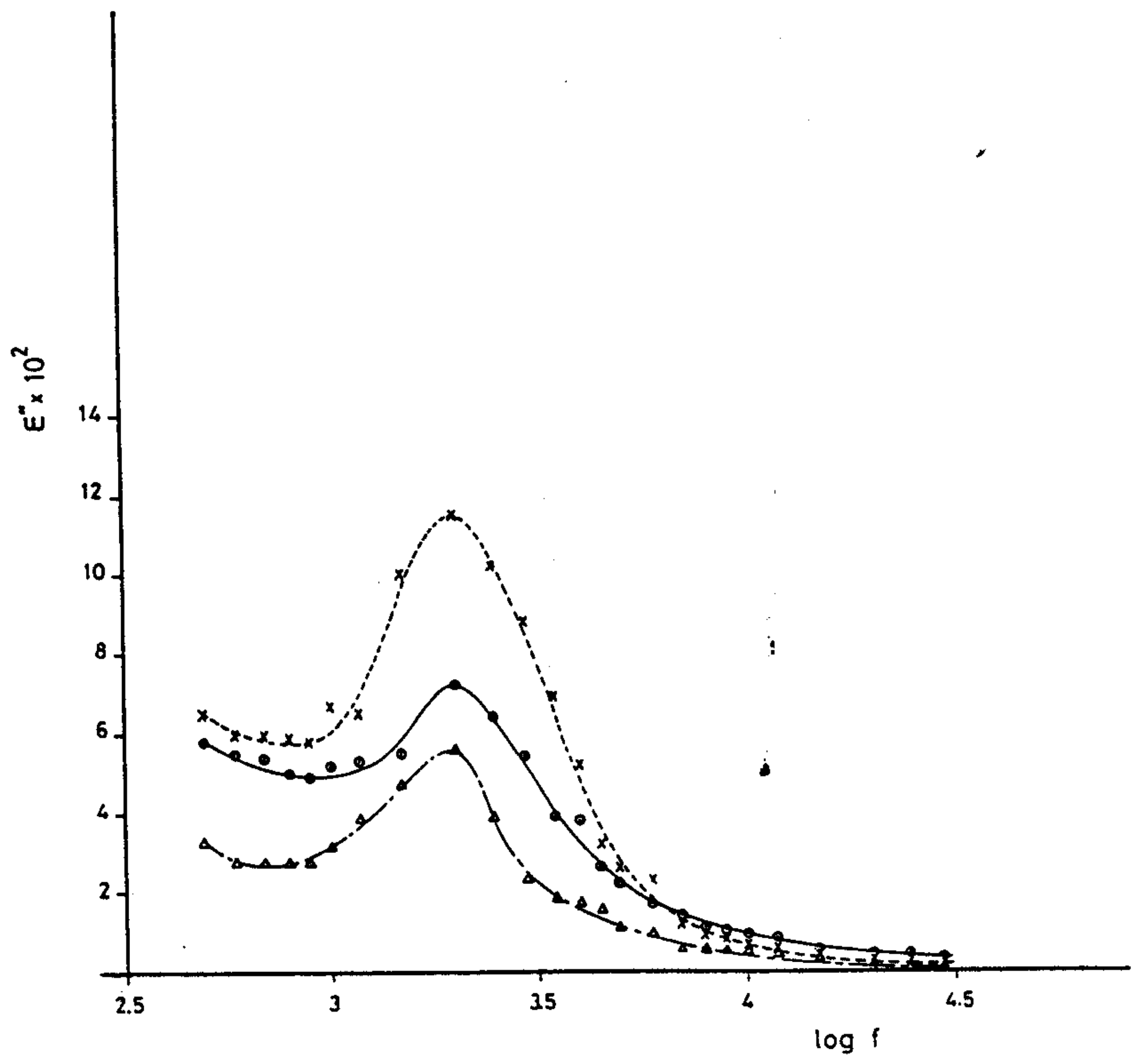

Fig. (4.18)a The relation between the dielectric loss (ϵ'') and the logarithmal frequency (log f) of dark adapted chicken eye exposed to laser beam of duration 0.12 at constant temperature 10°C for control, immediate and the fourth recovery day control (⊙), immediately (x) and the 4th recovery day (Δ).

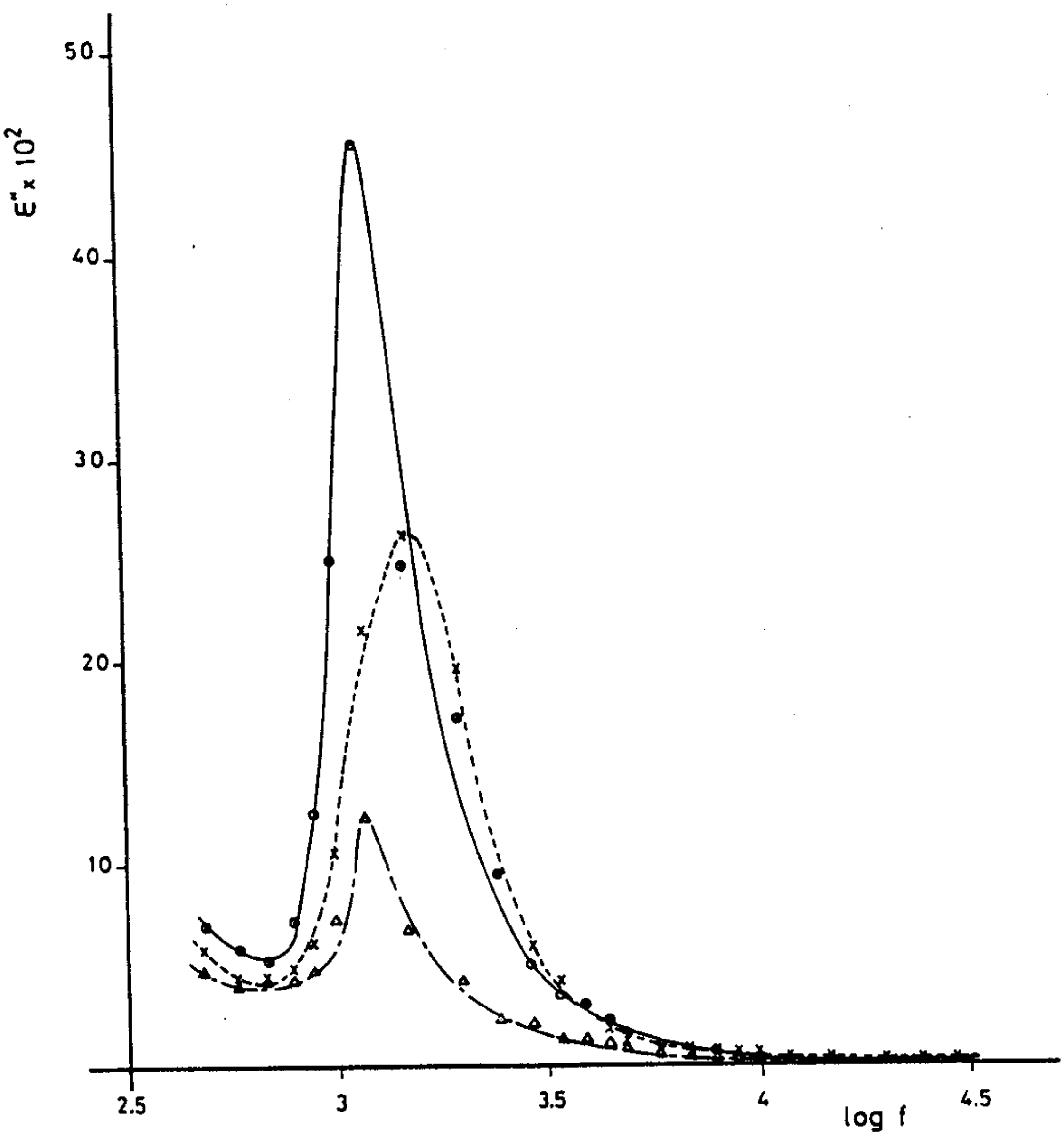

Fig. (4.18)b The relation between the dielectric loss (∈") and the logarithmal frequency (log f) of dark adapted chicken eye exposed to laser beam of duration 0.12 at constant temperature 20°C for control, immediate and the fourth recovery day, control (⊙), immediately (x) and the 4th recovery day (Δ).

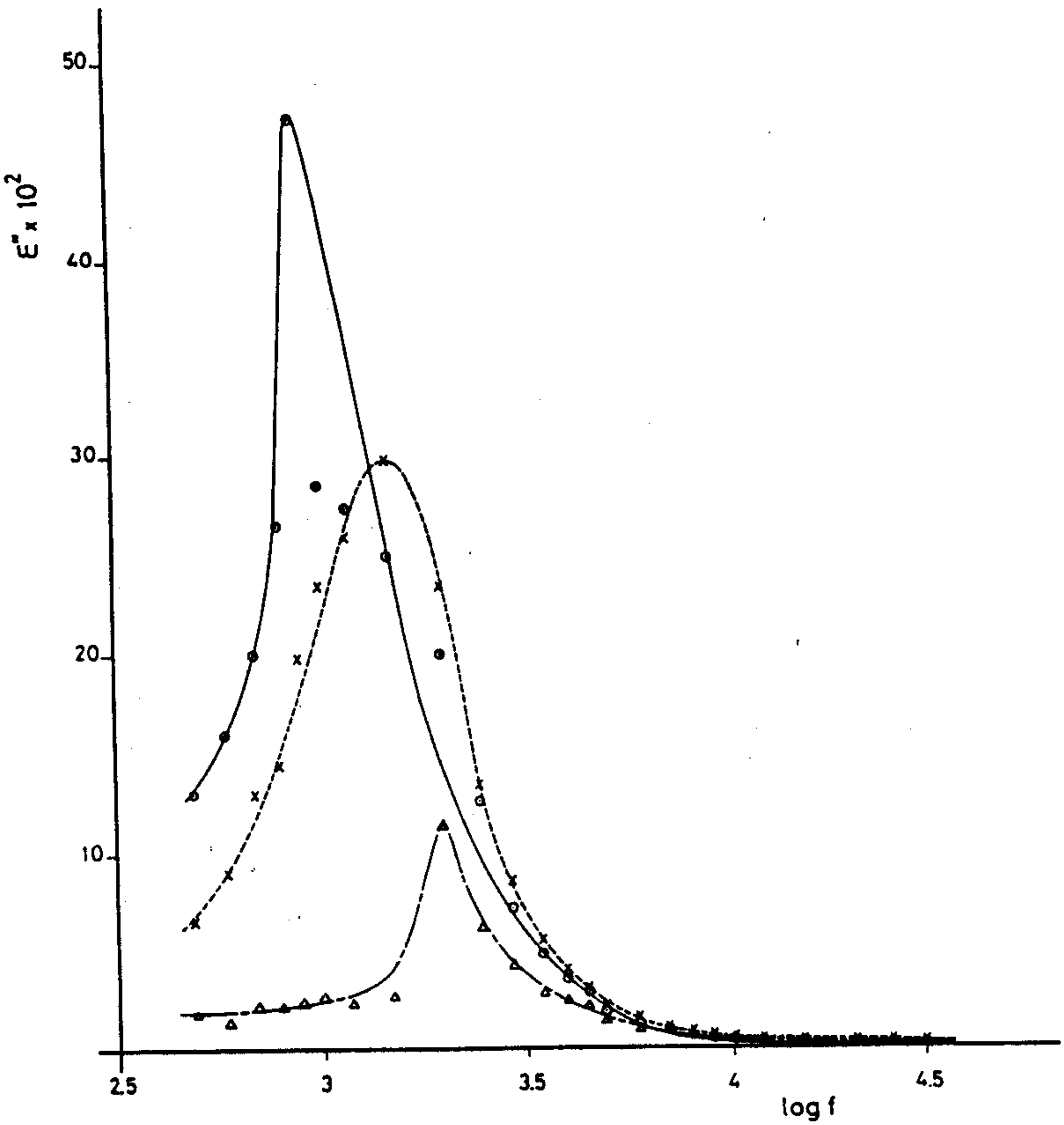

Fig. (4.18) c The relation between the dielectric loss (∈") and the logarithmal frequency (log f) of dark adapted chicken eye exposed to laser beam of duration 0.12 at constant temperature 30°C for control, immediate and the fourth recovery day control (⊙), immediately (x) and the 4th recovery day (Δ).

Exposure time 0.12 sec.

Temperature	10°C				20°C				30°c			
Conditions and recovery days	Δ ε'	θ	Fs	τX10^{-5}	Δ ε'	θ	Fs	τX10^{-5}	Δ ε'	θ	Fs	τX10^{-5}
N	779.4	7°8	2000	7.9	644	8°8	1200	13.2	1373	8°8	1500	10.6
I	812.4	8°7	2000	7.9	666.2	8°6	1500	1.06	669.1	8°8	2000	7.9
1	866.5	7°8	1200	13.2	1686	7°0	1000	15.9	719.2	7°9	900	17
2	1025.8	7°4	900	17	636.7	8°8	2500	6.3	588.3	8°8	2000	7.5
3	1635.6	8°7	900	17	1013.9	8°6	2000	7.9	949.1	8°8	3500	4.5
4	784	8°4	2000	7.9	725.1	8°5	1200	13.2	596	8°8	2000	7.9
5	937.3	7°5	1500	16.6	919.6	7°8	2500	6.3	919	8°4	2500	6.3
Exposure time 0.25 sec.												
N	779.4	7°8	2000	7.9	644	8°8	1200	13.2	1373	8°8	1500	10.6
I	837.1	8°0	2000	7.9	538.2	8°9	1200	13.2	662.4	8°7	800	19
1	748.6	7°9	700	22	795.8	7°3	800	19	719.2	7°8	700	22
2	1091.9	8°0	1500	10.6	642.6	8°6	2000	7.9	587.7	7°8	1500	10.6
3	954.9	8°0	2000	7.9	1013.9	8°6	2000	7.9	949.1	7°5	3500	4.5
4	2482.5	8°6	1000	15.9	772.2	8°3	1200	13.2	593	7°8	2500	6.3
5	890.1	6°9	1200	13.2	919.6	7°8	2500	6.3	884.3	7°9	2000	7.9

Table (4.5): The values of the dielectric parameters (Fs and T) of the dark adapted chicken eyes placed at constant temperatures (10,20 + 30°C) in normal conditions (N) and the five recovery days from the immediate (I) exposure to argon laser of constant intensity and duration (0.12 and 0.25sec).

parameters in both exposure time. This may be due to the lack of sensitivity of the dielectric methods specially at low frequency as stated by **Singh et al. (1979).**

In order to study the repairing processes (recovery) after the laser exposure, the relation between the maximum dielectric loss ϵ'' and the recovery days, after laser exposure of duration 0.12 sec and at fixed temperatures of 10, 20 & 30 °C is illustrated in Fig. (4.19).
It is clear from this Figure that the dielectric loss drastically decreases with the days after exposure but it increases again in the fourth day indicating an increase in the repairing process which could take place to overcome the effect produced by the laser beam.
It should be noted that the variations of maximum ϵ'' at 10 °C is less than those at temperatures 20 & 30 °C so that we may expect that this temperature is very suitable in decreasing the laser damage and hence in decreasing the time for complete recovery.
Regarding the changes in maximum ϵ'' with the recovery days at laser duration of 0.12 we noticed that they were more pronounced than those in case of laser exposure duration of 0.25 sec. as shown in table (4.6).

In order to compare between the effects of the conditions of adaptation (dark or light) on the recovery processes after laser effect, the same above experiments were repeated on light adapted chicken eye. The variations of maximum dielectric loss ϵ'' at different temperatures

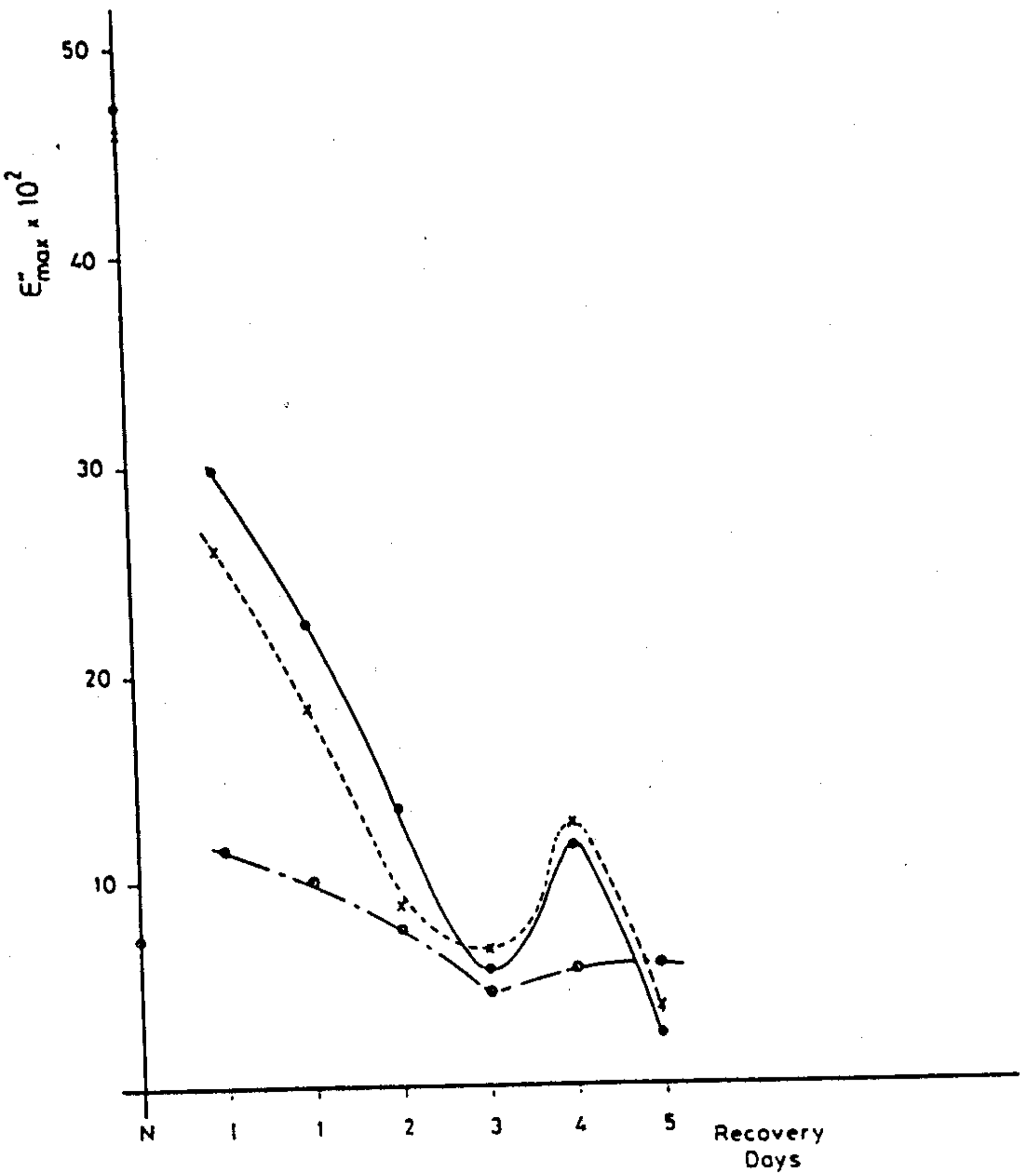

Fig. (4.19) The relation between the maximum dielectric loss ($\in''$) and recovery days of dark adapted chicken eye exposed to laser beam of duration 0.12 sec at constant temperatures of 10°C, 20°C and 30°C.
(⊙ — 10°C, x — 20°C and • — 30°C).

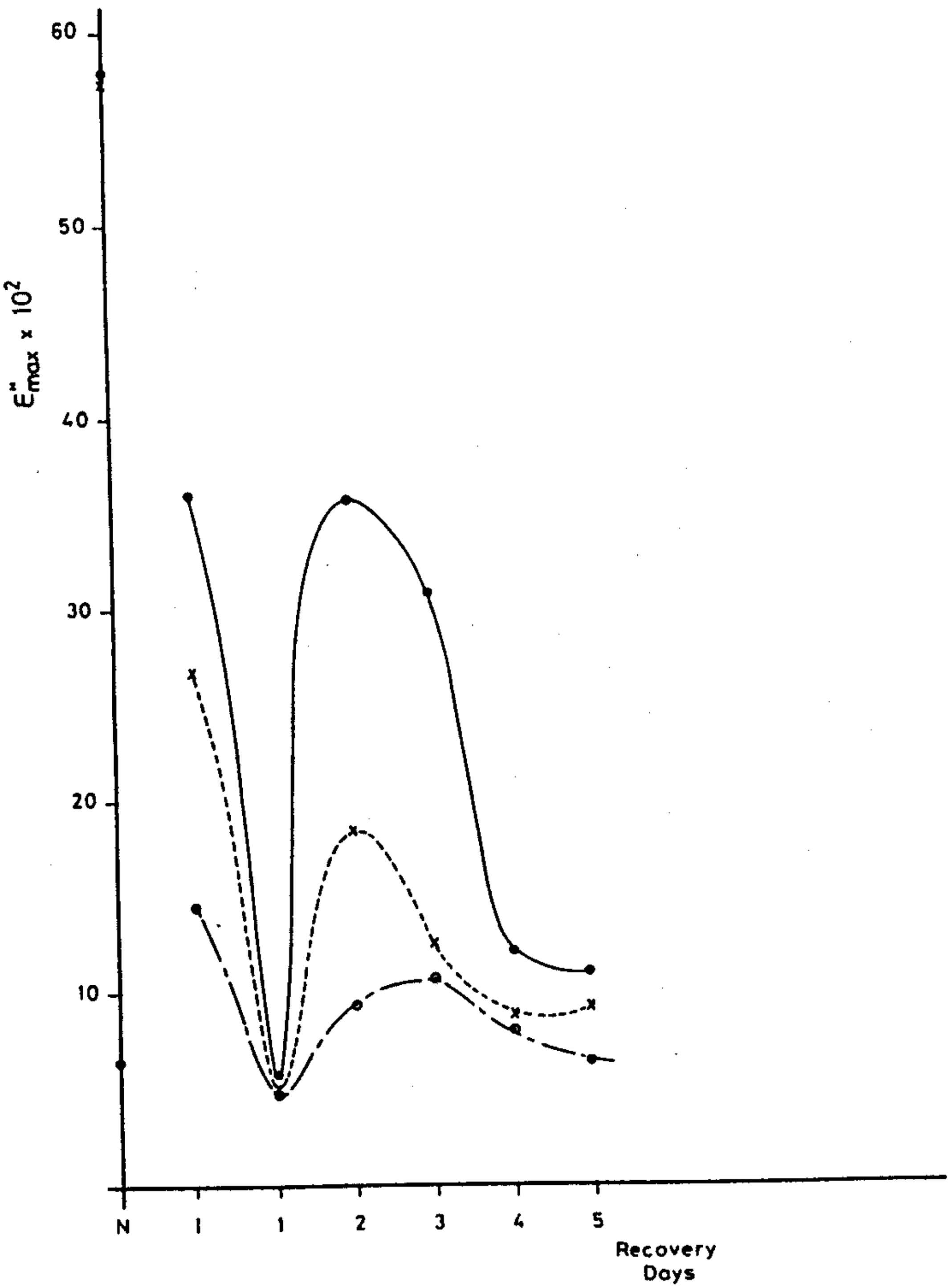

Fig. (4.20) The relation between dielectric loss (∈") and recovery days of light adapted chicken eye at constant temperatures of 10°C, 20°C and 30°C exposed to laser beam of duration 0.12 sec (⊙ — 10°C, x — 20°C and • — 30°C).

	0.12 sec			0.25 sec			
	Maximum dielectric loss (ε')						
Days	10°C	20°C	30°C	10°C	20°C	30°C	Treated
N	720	4600	4720	720	4600	4720	
I	1150	2630	2980	786	2780	2820	
1	1000	1840	2240	646	2654	252	D.A
2	760	880	1340	560	992	308	
3	450	660	560	665	672	551	
4	560	1270	1160	1490	926	554	
5	580	380	240	569	398	407	
N	649	5750	5800	649	5750	5991	
I	1450	2680	3600	1289	1600	1891	
1	470	500	580	631	1156	1446	L.A
2	940	1840	3570	1451	847	1451	
3	1070	1260	3080	1198	2322	3340	
4	800	688	1210	497	835	1500	
5	640	923	1100	551	923	505	

Table (4.6): The relation between maximum dielectric loss ε and recovery days after laser exposure of durations 0.12 and 0.25 sec of dark and light adapted chicken eyes at temperatures 10,20 + 30°C.

with the days after exposure of both durations are shown in table (4.6) and only represented in case of laser duration 0.12 sec in (figure 2.20). It is clear that the variations in ϵ'' of fig (2.19) i.e. in case of dark adaptation are more regular than that of fig (2.20) in case of light adaptation but generally, the processes of recovery are more pronounced at temperature 10°C in the fifth recovery day

Dielectric relaxation may be alternatively assumed to be a process where-by the rotating unit moves between two equilibrium position separated by a potential barrier. According to this picture, the relaxation time will be a measure of the number of times per second that such a process will occur and, accordingly, Eyring **(Glasstone et al., 1941)** has derived this expression.

$$\frac{1}{\tau} = \frac{K\tau}{h} e^{-\Delta F/RT} = Ae^{-\Delta F/RT} \qquad (4.5)$$

where ΔF is the molar free energy of activation and the other symbols have their usual significance.

From thermodynamic considerations

$$\Delta F = \Delta H - T\Delta S \qquad (4.6)$$

where ΔS is the entropy of activation and ΔH is the enthalpy (or heat) of activation. Since the variation of A with temperature is much less than the exponential term, a plot. of ln τ against 1/T produces an approximate straight line whose slope provides ΔH and whose intercept gives ΔS .These two quantities can then be related to structure since ΔH is a measure of bond strength and ΔS is related to local disorder.

As mentioned above the measurements of the relaxation time can be used to give an information about the changes in the structure and function of both dark and light adapted eyes especially when subjected to argon laser of constant intensity and different duration immediately after exposure and several days after it. The previous results described before, can be used to get the relaxation time from values of the relaxation frequency (table 4.7).

Fig (4.21) shows the main difference between the logarithm of relaxation time and reciprocal of temperature for both light and dark adapted chicken eyes. It is clear that the two straight lines intercept the ordinate at different parts. This indicates that the degree of disorder in case of light adapted is more than that of dark adapted which is in good agreement with the principles of photosensation as described before. The slopes of two lines approximately have the same values and it could be indicated that during light adaptation the general structure of the different components of the eyes approximately showed no reasonable changes. We conclude that, the relaxation time in both cases decreases with the temperature increase and the slope (heat) in both cases is approximately equal.

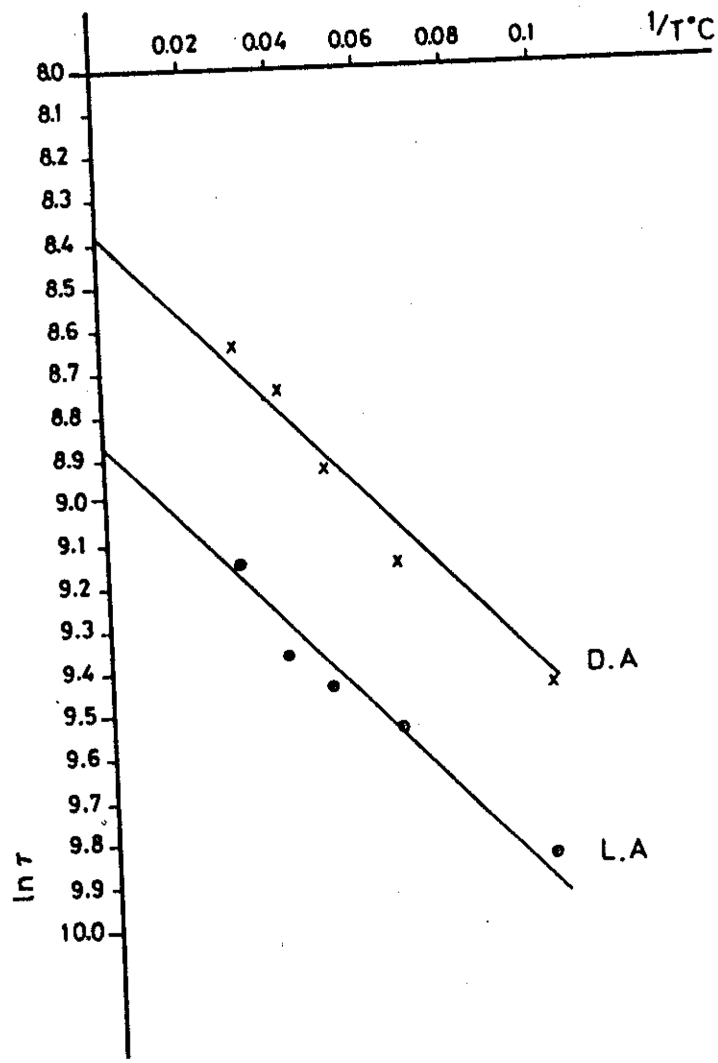

Fig. (4.21) The relation between the natural logarithm of the relaxation time (ln τ) and the reciprocal of temperature 1/T °C of light (L.A) and dark (D.A) adapted chicken eyes at normal condition.

		Light adapted		Dark adapted	
T°C	1/T°C	τx10^{-5}sec	Ln τ	τx10^{-5}sec	Ln τ
10	0.1	5.3	- 9.84	796	- 9.43
15	6.66	6.36	- 9.66	10.6	- 9.15
20	0.05	7.96	- 9.43	13.2	- 8.93
25	0.04	8.84	- 9.33	16.9	- 8.74
30	0.03	10.6	- 9.15	17.6	- 8.64

Table (4.7) : The values of the relaxation time τ sec of light and dark adapted chicken eye placed at different temperatures T°C.

From the above mentioned results, it is clear that the dielectric measurements indicated the laser sensitivity of the eye, especially at high doses. Since retinal laser treatment is now always performed, there may be some associated discomfort and some risks **(Frank, 1980)**. One of the major ophthalmic problem which occurs in case of diabetic retinopathy is the proliferation of a new blood vessel due the requirement for more oxygen by the retina. This can lead to a deterioration of vision and ultimately to loss of sight. This condition is now treated by pan-retinal laser coagulation where by the fundus of the retina receives a few hundred laser burns. The success of the treatment is not due to direct sealing of individual vessels as was originally imagined. Laser coagulation destroy a large area of retina and thus eliminates the requirement for more blood vessels. Thus a significant proportion of peripheral vision is sacrified in order to preserve central vision.

The results of this work showed that the dielectric method was proved to be a very sensitive tool in testing the functional state of the chicken eye during laser exposure. For example the decrease in the values of the maximum dielectric loss in the first days after laser exposure and the consequence increase of it during the fifth recovery day could be discussed as an increase and decrease in the retinal cell membrane permeabilities respectively. Also it could be due to vasodilation of the retinal blood vessels which is accompanied by an increase in capillary permeability. This permeability increase permits larger serum proteins to inter the cell, shifting the osmotic pressure at the capillary wall such that cell swelling takes place due to the interance of water. Other while, the repairing processes may be occurred due to that some intracellular substances released and gradually diffused out of the cells and this would explain the latent period of several days. This could be similar to that occurred in sunburns (erythema) as reported by **Sliney and Wolbrasht, 1980.**

Accordingly, we could define the concluded best condition for the application of argon laser in retinal treatment in order to reduce the critical ratio of damage/ repair which is extremely small by comparison with many other medical and surgical therapies. Such conditions are; the same as stated in the previous section that is laser application should be carried out in dark adapted conditions and at low temperature.

IV.3- Laser effect on IR retinal spectra :

It is difficult to study the effects of laser on the molecular structure of the chicken retina invivo state using the infrared spectroscopic technique without a lot of distortion and denaturation which might occurred during the procedure of study.

In this work a new sample preparation method was used, for,the I.R. spectroscopic study, in which the sample was first immersed into a biological fixer which would provide bonds that will hold the molecules together. Fixation forms crosslinks, not only between the reactive groups of the fixer and the reactive groups in the tissue, but also between the different reactive groups in the tissue itself. Gluteraldehyde is the most effective of the alldehyde fixatives for preserving fine structures. It stabilizes structures and prevents distortion during processing. In addition no other fixative surpasses gluteraldehyde in its ability to crosslink protein **(Elaine, 1984)**. The stability of protein (rhodopsin) inside the retina due to the fixative effect of the gluteraldehyde, enables us to study the structure and orientation of the rhodopsin inside the chicken retina at normal condition and after the laser exposure of the dark and light adapted retinii.

Fig. (4.22) shows the infrared retinal absorption spectra of normal light adapted chicken retina at constant temperature (20 °C) in the spectral region from 4000 to 200 cm^{-1}.

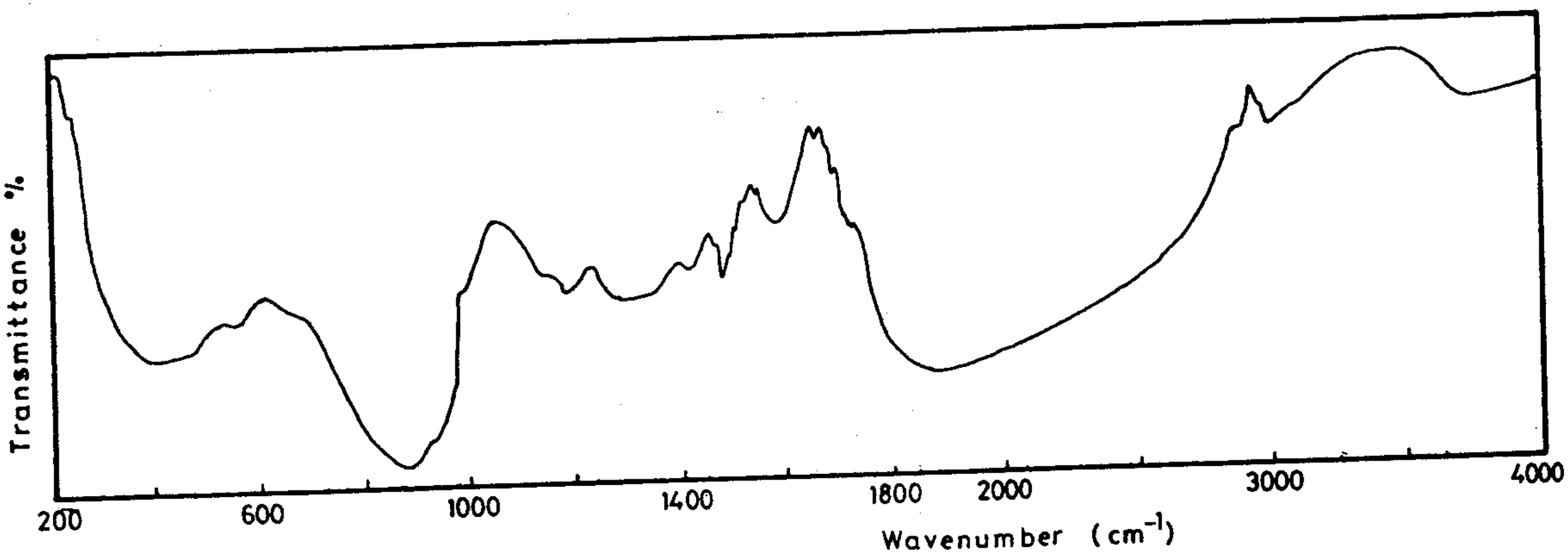

Fig.(4.22) The infrared spectra of normal light adapted chicken retina at constant temperature 20 °C.

The major peaks are assigned to either vibration of the protein part (rhodopsin) or the membrane lipids, as shown in table (4.8), together with that of purple membrane by **Rothschild (1979)** for the sake of comparison. The two peaks at 1664 cm^{-1} and 1549 cm^{-1} are assigned to the amide I which represent C = O stretching vibrations and amide II which represents the in-plane N-H bending vibrations **(Wallach et al., 1968 and Susi, 1969)**. Several other peaks in Fig. (4.22) can tentatively to either lipid or protein groups in the retina as shown in table (4.8) **(Champman, 1979)**.
The prominent peaks appeared at 1235 cm^{-1} and 1057 cm^{-1} are assigned to P = O (Phosphorus oxygen) stretching vibrations and P - O - C (Phosphorus, oxygen, carbon) stretching vibrations, which likely to be part of the phospholipid content of the retina **(Akutsu et al., 1975)**. Table (4.8) also shows that the assignment of the recorded infrared bands of the retina have approximately the same wave number as that recorded by polarized infrared spectroscopy for purple membrane **(Rothschild, 1979)**.

The appearance of amide I at 1664 cm^{-1} and amide II at 1549 cm $^{-1}$, in the I.R. spectra of light adapted retina at constant temperatures 10, 20 and 30 °C, indicates that the molecular structure of the protein is an α helix protein which is mainly rhodopsin **(Rotshchild, 1980, Osborne, 1977)**. In addition table (4.8) shows also that the absorption spectra of the retinal protein recorded by the present work is in a good agreement with that recorded by the polarized infrared studies of purple membrane deposited on different

Wave number (cm^{-1}) Chicken retina (I.R)	Purple membrane (polarized I.R)	Band Assignment
3436 (S) 3060 (Sh)	3310 3060	Amide A. Amide B.
2924 (S) 2852 (S)	2958 2850	CH strectching Vibrations.
1664 1549	1660 1547	Amide I Amide II
1452 1416 (W)	1457 1415	CH^3 bending Vibrations.
1384 1372	1382 1367	CH_2 stretching Vibrations.
1235	1235	P = O stretching Vibrations.
1150 (m) 1107 1057	1125 1100 1060	P - O - C Stretching Vibrations.
835 559	825 557	CH (rocking) Amid V amide VI (out of plane deformation).

Table (4.8) The absorption bands assignments of chicken retina and purple membrane. Symbols referring to the appearance of peaks are, S (strong) Sh (Shoulder), W (weak) and m (median). Peak frequencies were measured directly from spectra and found to vary more than ± 1 cm^{-1} for different samples.

types of slides or films using different methods **(Rothschild, 1979)**. Moreover the used technique is very simple and cheap with respect to other techniques using high technology and cost too much **(Powers et al., 1975 and Korenbrot, 1977,1979)**.

The proposed technique could be also used to study the spectra of the biological tissue in both vitro and vivo states depending upon the type of stimulus affecting the biological tissue under study. The recording of the absorption spectra of rhodopsin from the whole retina as used in this work is supported by the work of **Fumio et al. (1976)**, who proved that the visible and ultraviolet absorption spectra of the total retina and that of the extracted rhodopsin were the same.

To study the effect of light and temperature on the molecular structure of the rhodopsin inside the retina, the infrared spectra of light adapted sample placed at the same temperatures (10, 20 & 30 °C) are recorded. The obtained spectra are shown in Fig. 4.23, in which the absorption bands of amide I and amide II for light and dark adapted samples appeared at the same previously mentioned wave numbers, i.e. there is no noticeable frequency shift observed. However, the intensity of these characteristic bands, calculated by the use of **Beer's Lambert** law and base line method , showed a detectable changes as listed in table (4.9). It shows also that the intensity of the characteristic absorption bands increase with the increase in the temperature of the specimen in both cases of adaptation.

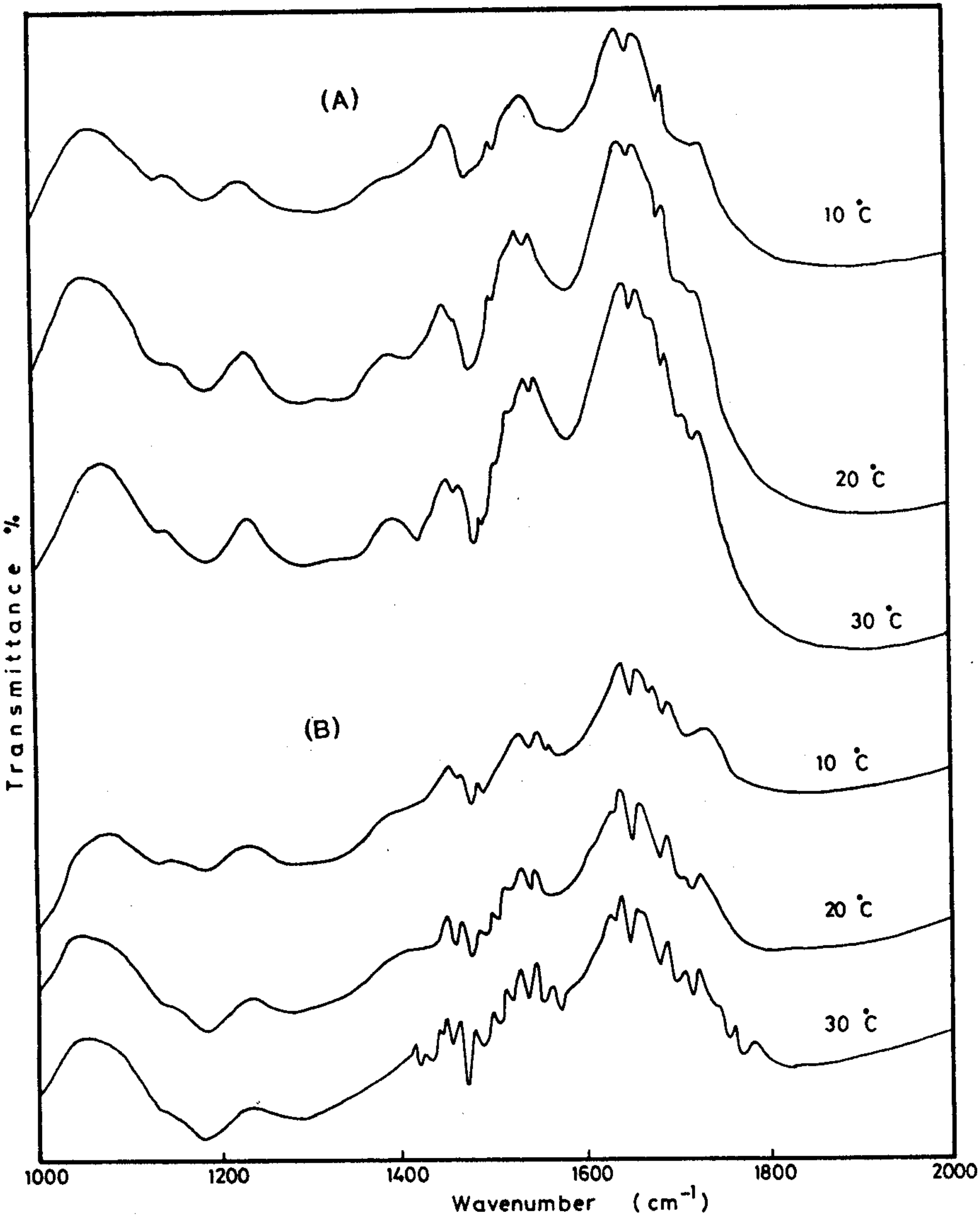

Fig.(4.23) The infrared spectra of light (A) and dark (B) adapted chicken retina at constant temperatures 10 20 and 30 °C.

Such results support the idea that when the temperature increases the permeability of photoreceptor membranes increases to the serum protein. Henc the intrance of that protein causes the increase in the intensity of the absorption bands as shown in table (4.9).

	Amide II 1549 cm^{-1}	Amide I 1664 cm^{-1}	Amide II / amide I	T°C
1- Light adapted	0.311	0.399	0.770	10
	0.452	0.653	0.692	20
	0.522	0.790	0.661	30
2- Dark adapted	0.465	0.590	0.788	10
	0.478	0.625	0.766	20
	0.490	0.720	0.680	30

Table (4.9) The absorption intensity of the characteristic bands and the intensity ratio of amide II and amide I for light and dark adapted retinii.

In proteins, where the polypeptide chain has a folded configuration (α- helix), the N-H stretching and the amide I bands have parallel dichroism while the amide II has perpendicular one **(Beer, 1958)**. The observed dichroic ratios of the several characteristic bands in proteins have been used to make quantitative test of the various polypeptide chain configurations which may exist in proteins. Since the amide II has perpendicular dichroism and amide I has parallel one, the ratio of the absorption intensity of amide II over that of amide I could be used to get information about the orientation angle of rhodopsine with membrane normal as previously given by **Rothchild (1979)**, who found a linear relation between the ratio of amide II/amid I and the purple membrane rhodopsine orientation angle as shown in Fig. (4.24). Since the absorption bands of amide II and amide I of purple membrane rhodopsin appear at the same wave numbers as that of chicken retina, so this relation could be exploited to define the orientation angle of rhodopsine with membrane normal protein placed at different conditions.

According to the present work experimental data given in table (4.9) and that of **Rothschild (1979)**, one could define the orientation angle of rhodopsine with membrane normal for light and dark adapted chicken retina at temperatures 10, 20 and 30 °C which are 31°, 43° and 48° respectively and 30°, 33° and 35° respectively.

According to the present work results it is clear that the orientation angle of rhodopsin with the membrane normal for light adapted retinii is more than that of the dark adapted. These results could be explained as follows : when the light falls on the rhodopsine inside the retina, a series of photochemical reactions occur resulting in the splitting of the protein part of rhodopsin (opsin) from the bleached chromophore part as discussed in chapter (I). Also, changes occur in the opsin (protein part), which are only a reorientation of tryptophan residues of protein to be perpendicular to the membrane normal and hence causing a change in the total orientation of the protein with respect to the membrane plane **(Chabre, 1978)**. In dark the chromophore part goes through a path of isomerization process before the reattachment with opsin to form rhodopsin again.

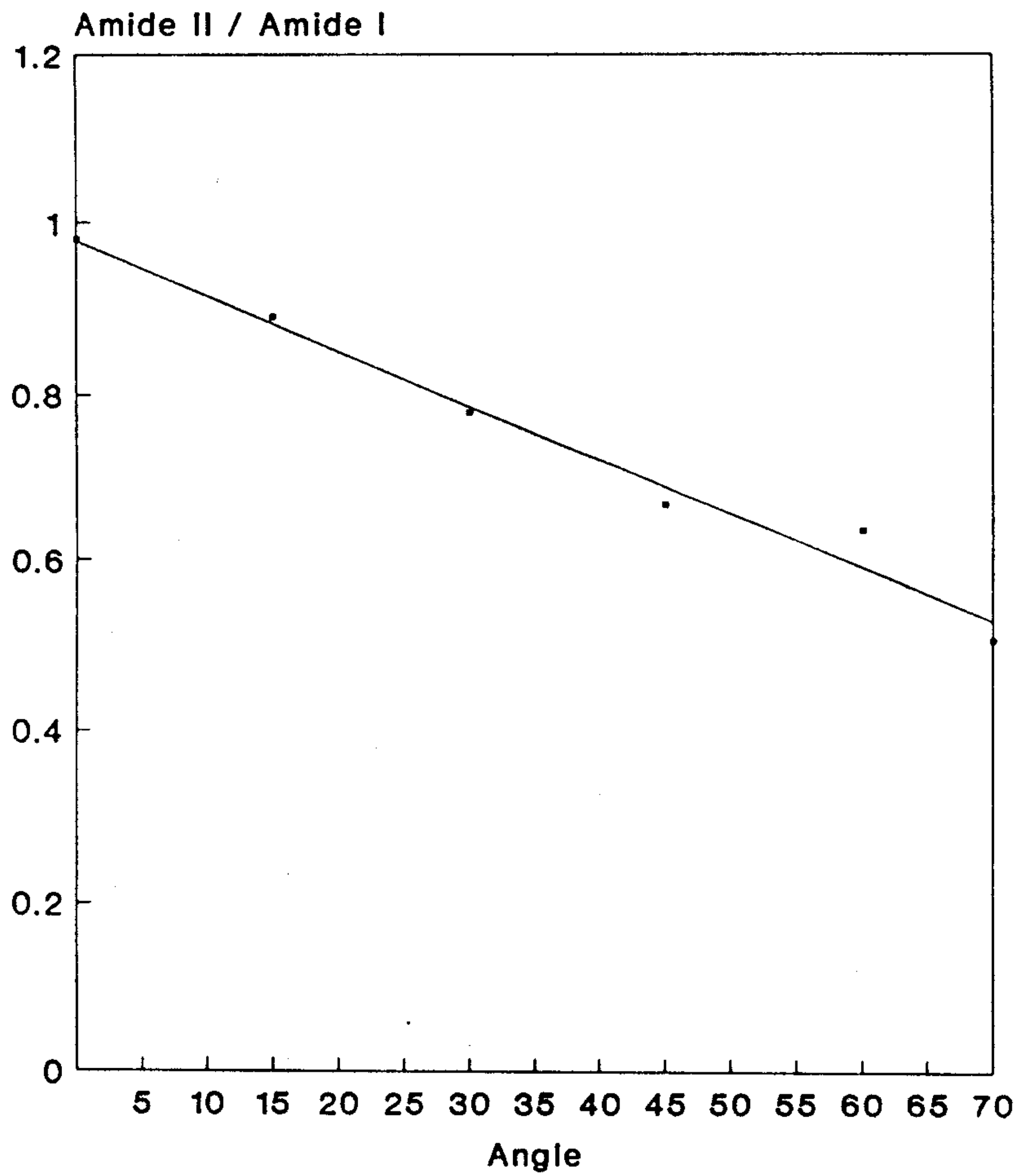

Fig. (4.24): The relation between the intensity ratio of amide II/amide I of purple membrane and the rhodopsin orientation angle (Rothschild, 1979).

The above mentioned technique was used to study the molecular structure changes of dark and light adapted chicken retinii placed at different temperature and exposed to argon laser of constant intensity and different durations. The experiments were carried out as follows, two groups of light and dark adapted chicken eyes from the same species and about 2 - 3 weeks age were exposed to laser of intensity 1 mW/cm^2 and duration 0.12 sec.

During the exposure, the bird was kept at constant temperature. (The used temperature are 10, 20 & 30 °C). After exposure a number of birds were left in the same condition of adaptation and the other were immediately decapitated for the IR spectrophotometric studies. The other lifted exposed birds were decapitated after 1 to 5 days in order to study the recovery processes which might occurred to compensate the destructive laser action.

Fig. 4.25 (a,b) represent the infrared spectra of light adapted chicken retina at constant temperatures (20 & 30 °C) and duration 0.12 sec, immediately and during (1 - 5) recovery days after laser exposure. Also, Fig. 4.26 (a,b) shows the infrared spectra at the same condition of Fig. 4.25(a,b), but at temperatures (10 & 20 °C) and duration 0.25 sec. Figures 4.25 & 4.26 (a,b) show that no new bands appeared in the I.R. spectra of retina due to the effect of laser beam and no frequency shift was observed in the position of the general amide characteristic bands. This indicates that there is no change in the main helical

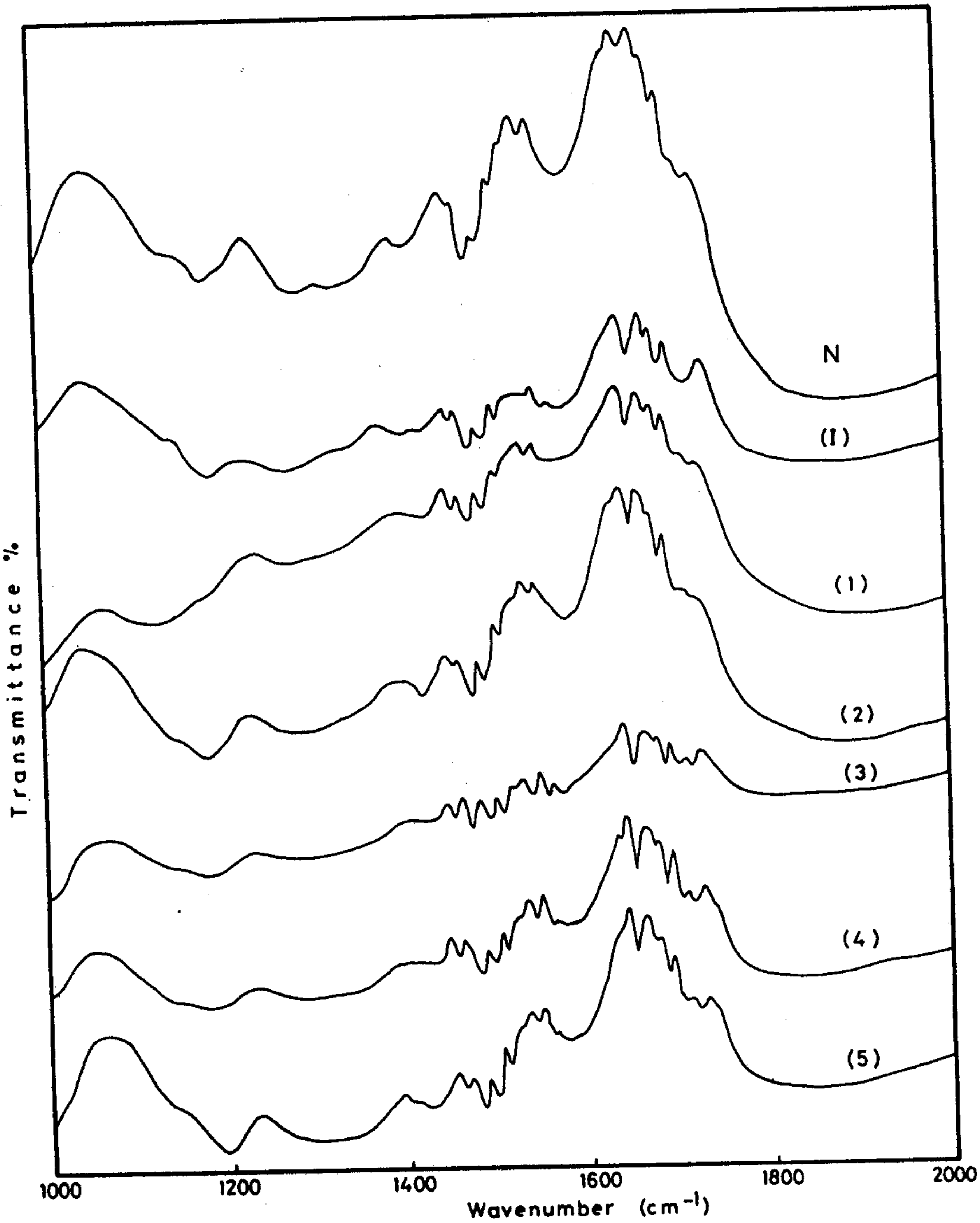

Fig.(4.25)a : The infrared spectra of light adapted chicken retina at constant temperature 20 °C and duration 0.12 sec during normal N, immediately I and (1-5) recovery days.

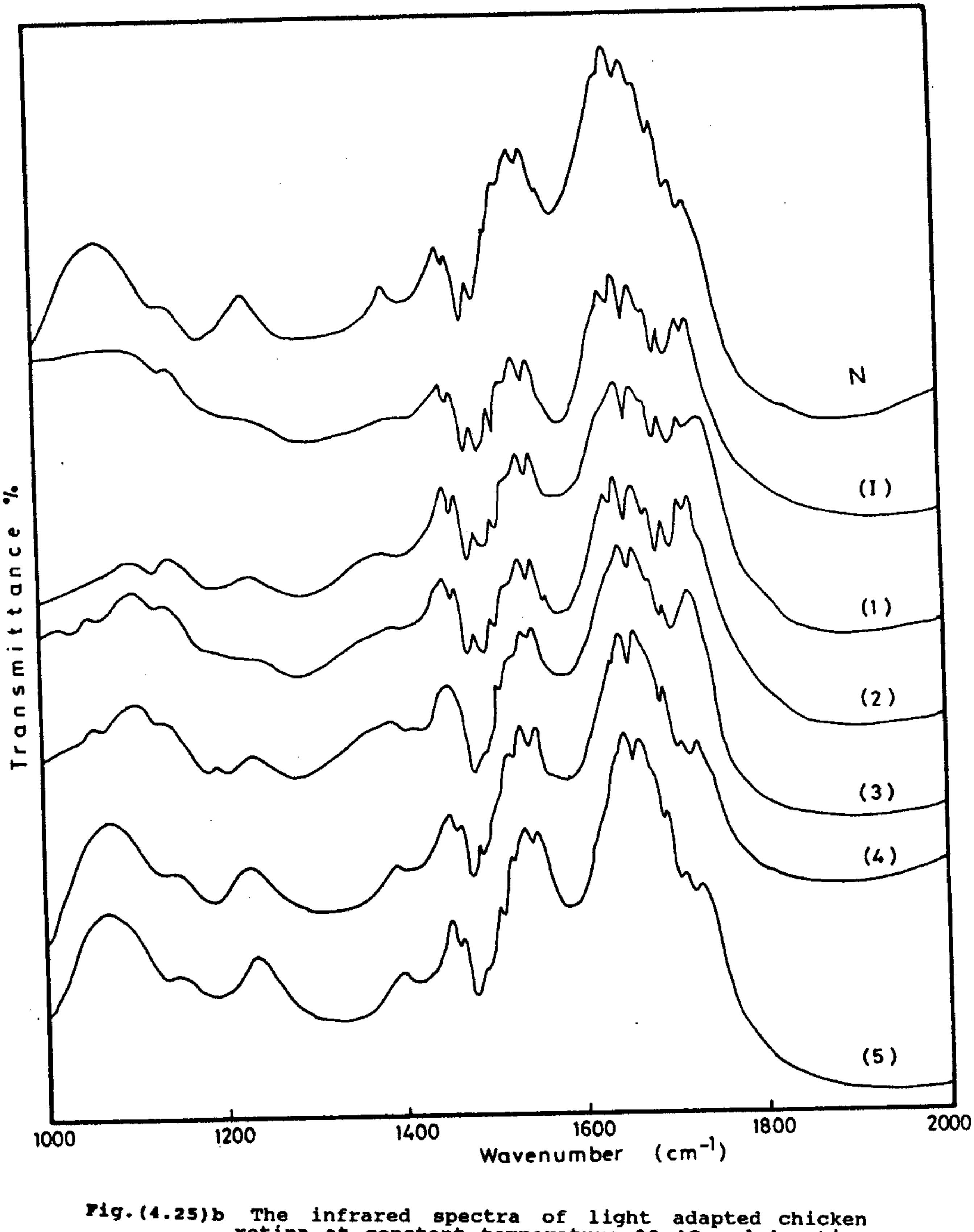

Fig.(4.25)b The infrared spectra of light adapted chicken retina at constant temperature 30 °C and duration 0.12 sec, during normal N, immediately I and (1-5) recovery days.