THE RHEOLOGY OF SKIN

A bio-engineering study of the mechanical properties of human skin in relation to its structure

M.D. Ridge

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Department of Medicine, University of Leeds

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1. INTRODUCTION

The present work has been carried out in the Rheumatism Research Unit of the University Department of Medicine at Leeds. In this Unit one of the major fields of investigation has been into the nature of joint stiffness.

Altered stiffness occurs in numerous disorders of connective tissue. For many years increased stiffness has been recognised as a major manifestation of joint diseases, such as ankylosing spondylitis (Forrestier, Jacqueline and Rotes Querol, 1951; Hart and McLagan, 1955; Blumberg and Ragan, 1956), rheumatoid arthritis (Short, Bauer and Reynolds, 1957), degenerative arthritis (Abrams, 1953), primary generalised osteoarthritis (Kellgren and Moore, 1952), and gout (Talbot, 1953). The term "articular gelling" has been applied to increased stiffness which is most troublesome after periods of rest in one position, noted by patients with different forms of arthritis (Hollander, 1953). Stiffness is usually accompanied by pain and is often considered to be due to muscular spasm associated with pain, although Taverner (1954) has clearly shown by electromyographic studies that these may not be related. In ankylosing spondylitis

Forrestier, Jacqueline and Rotes Querol (1951) found that 17.5% of their patients had stiffness without pain.

Patients with active rheumatoid arthritis commonly complained of waking in the morning with greatly increased stiffness which may persist for several hours. This is so characteristic that it has been termed "morning stiffness".

Investigating morning stiffness Wright (1959) measured the strength of grip of patients with rheumatoid arthritis throughout the day. He found a marked weakness in the morning which improved in the first three hours of the day. However, study of control subjects without arthritis disclosed a similar diurnal pattern.

Wright and Johns (1959) therefore devised an apparatus which they later modified (Johns and Wright, 1960) for the measurement of joint stiffness directly. They showed that the major physical components of joint stiffness were elasticity and plasticity. Frictional stiffness even in the most badly damaged joints was very small and viscous stiffness contributed only 10%. Except in neurological diseases such as Parkinsonism and myotonia congenita reflex muscle contraction played no part in the production of stiffness measured at the joint (Wright and Johns, 1960 and 1961).

In order to ascertain the proportions of stiffness produced by passive stretching of the muscles and periarticular tissue the wrist joint of the cat was investigated (Johns and Wright, 1962). Rheologically this was comparable to the metacarpo-phalangeal joint of a child. It was found that the periarticular tissue produced 50% of joint stiffness during the normal range of joint motion.

Certain hereditable disorders of connective tissue have well recognised alteration of joint stiffness. The most striking example from this point of view is Ehlers Danlos syndrome, which is characterised by hypermobility of the joints (Benjamin and Weiner, 1943) and increased extensibility of the skin (Burrows and Turnbull, 1938). The skin is often described as hyperelastic in clinical practice (Jansen, 1955; Zaidi, 1959). This is a confusion of terms which can be misleading resulting in a discussion of the pathological lesions of connective tissue in this disease in terms of the physical causes of hyperelasticity. In fact in physical terms the skin shows decreased elasticity (or elastic stiffness). The original description of Job van Meekeren (1682) of a man with "extraordinary extensibility of the skin" (figure 1) is more accurate than the modern tendency to talk in terms of hyperelasticity. Other hereditable disorders of connec-

FIG. 1

JOB VAN MEEKEREN'S CASE OF EHLERS-DANLOS

tive tissue showing decreased joint stiffness are Marfan's syndrome (McKusick, 1956) and osteogenesis imperfecta (Slott and Burgess, 1937). Patients with the Hurler's syndrome on the other hand often demonstrate striking limitation of extensibility of joints (Hubeny and Delano, 1941).

Having established that alterations of stiffness at the joint could be caused by changes in the periarticular structures independent of muscular attachments, it became desirable to devise means of studying the rheology of the connective tissue, which makes the major contribution to this stiffness. In vivo studies of connective tissue are limited and it was therefore decided to undertake a rheological analysis of skin in vitro, since this is the most readily accessible source of connective tissue. It is well known that in connective tissue disorders, such as rheumatoid arthritis, the connective tissue is profoundly affected throughout the body, including the skin (Short, Bauer and Reynolds, 1957).

Considerable advance in the knowledge and structure of rubber and plastics and other polymers has been made through similar studies of their elastic and plastic properties. This has been convincingly demonstrated in

the study of the creep behaviour of a series of butyl rubbers, differing in molecular architecture (Alfrey and Gurnee, 1957). Connective tissue in normal and pathological situations studied similarly may well yield information about alteration in elastic elements (collagen and elastin), alteration in their cross linking or wicker-work and alteration in the viscous medium (ground substance). An extensometer for determination of the rheological properties of skin in vitro has therefore been devised and constructed, and a rheological analysis of skin has been made.

Some workers have attempted to describe the rheological behaviour of certain biological tissues by assuming some type of structure and then working from first principles mathematically in an attempt to derive an equation which would fully explain the behaviour of the tissue. The classical thermo-dynamic analysis of Frenkel (1946) and use of the kinetic theory of elasticity by Wall (1942 a and b) and Hall (1951 a and b) are typical examples of this. The mathematics of this type of investigation are of a high order and in some cases are such that it would be impractical or far too tedious to use such methods in the analysis of experimental work. Moreover, no theoretical equation has been found which completely and accurately describes the behaviour of biological tissue.

This is because the structure is extremely complicated and has not been fully evaluated.

It was therefore decided to make a preliminary rheological investigation of skin and to attempt to determine an empirical mathematical equation which would characterise the results. Having done this it was possible to interpret the constants of the equation in terms of various properties of the material. By this means obvious physical differences in terms of specific components of skin could be detected and quantitative comparisons could be made.

Using the approach of the "integrative school" of rheologists (Scott Blair, 1949) the rheological properties of skin have been characterised mathematically. The complexities of measurement have been explored and methods designed to accomplish this. The effect of physiological factors on the determined constants and their significance related to the observed structure of skin have been described. Some data on the effect of disease states will be presented.

11. SURVEY OF PREVIOUS WORK.

A. A CRITICAL ANALYSIS OF RHEOLOGICAL TECHNIQUES AS APPLIED TO BIOLOGICAL TISSUES.

The analysis of the results of various workers who have investigated the rheological properties of connective tissue is difficult. This is not due to lack of workers in the field, but because there is no standardised or generally accepted method, and consequently each investigator has developed his own technique, so that results are not directly comparable. The various techniques used can however be classified into groups which depend on the same fundamental principles.

1. Constant rate of stress
2. Creep and constant stress
3. Constant rate of strain
4. Stress relaxation
5. Impact
6. Dynamic
7. Special and empirical

1. Constant rate of stress.

This technique is one of the most popular in the field of biological rheology. In principle the specimen is subjected to a force which increases at a constant rate and the corresponding deformation is recorded. It is then assumed that the stress (usually tensile or compressive) which exists in the specimen in the direction of the application of the force increases at a constant rate. This assumption is only valid if the deformation of the specimen is small, as a large deformation will produce a change in cross-sectional area, which will cause an inaccuracy in the value of the stress. If the value of Poisson's ratio is known for the material, some form of compensation for the change of area can be made. Unfortunately many workers have disregarded this and carried out experiments on tissue, the deformation of which is sometimes excessive, without modifying the results to compensate for this source of error (Krafka, 1937; Green and Loughborough, 1945).

If results are required for comparison, and not for theoretical mathematical analysis, the use of large deformations can be justified, providing that all specimens are deformed by approximately the same amount.

Consequently although there would be an error in the stress value, it would be the same for all specimens.

The simplest type of constant rate of stress system is the form used by Green and Loughborough (1945) in which the specimen was hung vertically between two small jaws. A cord from the lower jaw passed over a pulley to which a pointer was fixed; this moved over a scale to give indications of the extension, and the cord was then fastened to a small bucket. The constant rate of loading was achieved by letting small lead pelletts slide down a shute into the bucket, thus increasing its mass and hence the load on the specimen. This system, whilst being adequate for simple investigations, could not achieve any high degree of accuracy. One reason for this was the difficulty in controlling the rate at which lead pelletts were dropped into the bucket. A means of overcoming this would be to substitute some liquid, for example mercury, in the place of lead pelletts; the flow could then be controlled accurately by means of a small needle valve, to achieve a smooth and predictable rate of loading. Apparatus utilising this general principle has been used by Krafka (1937). He recorded extension automatically with a smoked drum and pointer instead of the scale. This

eliminated the tedious, inaccurate task of taking manual readings.

A similar apparatus was also used by Harkness and Harkness (1959) in their excellent studies on changes in the uterine cervix of the rat. They used a smoked drum for recording extensions. Weights were applied manually at the rate of 25gm. every 15 seconds. This resulted in a stepped stress strain curve but the effect was small enough to avoid interference with the results.

A popular method of achieving a constant rate of increase of load has utilised a length of light chain hanging in a loop, one end of which is coupled to the specimen via a lever, pivotted at its centre, and the other is wound round a drum which is rotated at a constant speed, thus increasing the length, and hence the weight of chain hanging in the loop. This method was used by Hall (1951) and provided the basis of some very impressive work on collagen fibres. A similar, but rather more refined method was used by Morgan (1960) who also worked with collagen fibres. His apparatus was capable of testing eight specimens simultaneously. Each was fitted with its own chain loading system on a common winch shaft. Two small metal plates were fitted parallel to each other,

one to each jaw at the end of the specimen. Separation between the plates increased as the specimen was extended, thus forming a simple variable capacitor. An electronic instrument was used capable of scanning the eight capacitors in a pre-determined sequence, and interpreting the results as extensions of the various specimens. This system was reported as being accurate to within 0.01mm. and was used on specimens 0.6cm. long and loaded at the rate of 6 grams per minute.

An ingenious system was used by Conabere and Hall (1946). The apparatus consisted of a tiltable platform fitted with a column at right angles to it. From the top of the column a pendulum was suspended and the specimen was mounted between small projections on the pendulum and column in such a way that when the platform was horizontal and the pendulum vertical there was no tension in it. On tilting the platform a tension was produced in the specimen due to its pulling the pendulum from vertical. Consequently by controlling the angle of the pendulum to the vertical by tilting the platform it was possible to induce a constant rate of loading in the specimen tested. This however had disadvantages in that it was non-recording and the constant rate of loading was produced manually. Furthermore, no provision was made

for testing specimens under liquid.

Numerous writers (Hunter, 1936; Krafka, 1939; Crompton, 1949; Roddy, 1952) have with varying degrees of success used a commercially produced instrument known as the Scott incline plane serigraph (described in detail by Booth, 1961). This instrument produces a constant rate of loading and is used mainly in the textile industry. It consists of a tiltable platform on which runs a small trolley. The specimen is mounted between the end of the platform and the trolley. If the platform is tilted in a suitable manner mechanically, a constant rate of loading can be achieved. The stress strain graph is drawn automatically. This is a useful apparatus but for biological work is limited because the specimen cannot be tested under liquid.

One of the pioneers in the study of the rheology of biological tissue was Roy who worked with beautifully constructed equipment and despite the earliness of his work produced some extremely interesting results in connection with the elasticity of arterial wall (Roy, 1880). A specimen of artery was distended by increasing the internal pressure with a variable mercury column. The volumetric distension of the specimen was manifest as a volume of fluid displaced. This was detected by a dia-

phragm, the deflection of which was amplified by a lever and produced a recording on a smoked drum.

An interesting micro-extensometer was developed by Probine (1959) and used on plant cell walls. The principle of this machine was as follows:- a long arm was fixed to a pivotted vertical shaft A and was free to rotate in a horizontal plane. A flat spiral spring had its inner end fixed to the shaft and the outer end driven about A as an axis, by a synchronous motor operating through a worm drive. As the free end of the spring was driven from its rest position the torque on the shaft A was increased at a constant rate and tended to cause a displacement of the arm. The test sample being mounted between a fixed pillar and a point on the machine arm prevented the movement of the arm, and a constant increase in torque therefore resulted, the specimen being loaded at a constant rate. The extension of the specimen was measured by a capacitance system connected to the horizontal arm. The signal from the capacitance system was fed via an amplifier to chart recorder, the chart of which was driven by the same synchronous motor that drove the free end of the spiral spring. This system has much to commend it but is limited to small specimens, due to

the size of the control spring and the torsional forces necessary.

No reference has been found in the literature to the use of an instrument which could load at a constant rate and yet compensate for the reduction in area. Since large extensions were often reported many results obtained must be treated with reservation.

2. Creep and constant stress.

The creep testing technique is probably the most simple in the field of rheology, but has been used by relatively few workers for tests on biological tissue (Roy, 1880; Probine, 1959). In this system the specimen is subject to a constant force and the change of deformation recorded as time progresses. The specimen is usually mounted vertically between two jaws and some mass is attached to the lower one. Many systems have been adopted for recording the extension, which is usually very rapid at first and diminishes to an almost imperceptible amount after a certain time. This technique however is subject to the same criticism as the first in that the area will change for large extensions and consequently results in a higher stress. The principle of the constant stress system is identical to that of the creep test.

However, some form of compensation is built into the apparatus, so that the deforming force alters to produce a constant stress as the cross-sectional area of the specimen changes.

Probine (1959) working with plant cell walls used a micro-extensometer in which the extension was applied by a constant mass suspended in liquid. Extensions were recorded by a low speed cine camera observing the specimen through a microscope. This system whilst fulfilling the basic requirements has the disadvantage of not presenting the information in the most useful form, since a stress time curve still has to be plotted manually. The early work of Roy (1880) involved a useful piece of apparatus consisting basically of a horizontal lever pivotted at one end. The lower end of the specimen was attached to a point on the lever near the pivot. The upper end was anchored to a point vertically above. The stress was applied by a mass hung from the lever. A stylus was attached to the free end of the lever and recorded extensions on a smoked drum revolving at a constant speed. The apparatus was limited to relatively short extensions, since the tension in the specimen became less as the lever fell further below the horizontal.

The true constant stress system while having considerable application has received little interest in the field of biological rheology, despite the fact that the technique has been used considerably in other areas. The fundamental problem of causing the force to vary with extension in such a way that the stress would remain constant is complicated; nevertheless numerous good approximations have been made. An example of this is the parallel plate Rheometer (Scott-Blair and Coppen, 1941; Scott-Blair and Veinoglou, 1943). The principle of this apparatus is that if a mass is suspended from a horizontal arm which is pivotted at one end, the torque produced by the mass about the pivot reduces as the arm falls below the horizontal position. Thus if the specimen is mounted vertically between some point on the arm and another fixed point above it, the tension in the specimen is reduced as it is extended. This interestingly enough is a principal of the apparatus used by Roy (1880), although the possibility of this being a constant stress system did not appear to be considered in his paper.

Other devices have been employed in the pursuit of a constant stress system, for example wires or filaments of material have been tested by suspending from a fixed support with a mass on the lower end (Andrade, 1910).

The mass is shaped in such a manner that if it is allowed to come into contact with a liquid, the tension in the specimen is reduced in proportion to the amount the weight is permitted to sink. An alternative system has been used in which the effective tension in the specimen is reduced by means of cams (Andrade and Chalmers, 1932), but with both these systems considerable difficulty is experienced in producing the theoretical shapes accurately. Nevertheless once satisfactory equipment has been developed, it should be relatively simple to use.

3. Constant rate of strain.

This technique is common in many fields of rheological testing such as engineering, textile and leather industries. It consists of extending the specimen at constant rate and observing the change in force necessary to produce this extension. The popularity of this system probably lies in the fact that with relatively simple apparatus accurate results may be obtained. Unfortunately, however, with nearly all biological tissue the results are dependent on the rate at which the specimen is extended. It becomes difficult and in some cases impossible to compare the results of specimens which have been tested at different rates. Consequently it is important that rates of extension should be standardised

and the value stated with the results obtained.

There seem to be few references to these extensometers in testing biological tissue, possibly due to the fact that specimens are small and so are the forces necessary to produce the extension, giving the impression that very precise equipment would be required. Nevertheless extensometers of this type can be made with extreme accuracy.

However, irrespective of the nature of the problem to be investigated, the various apparatuses work on the same basic principle. With apparatus of this type the specimen is usually mounted vertically between two jaws, the top one of which is connected to some load measuring system, usually mechanical or electrical. The bottom jaw, which performs the actual extending, is connected to a nut which runs on an accurately made screw thread. This screw is caused to rotate at a constant speed, consequently extending the specimen at a uniform rate. Both load detecting and extending systems are usually connected to some form of recorder on which the stress strain curve is produced.

The properties of skeletal muscle have been investigated by a system of this type by Schottelius (1957) who used an apparatus developed by Talbot, Lilenthal,

Beser and Reynolds (1951). The elasticity of elastin from ligamentum nuchae and its alteration by enzymes has been studied with our apparatus (Hall, 1964).

4. Stress relaxation.

In this mode of testing, the specimen is subject to a sudden deformation, which is maintained while the resulting load is observed to diminish with time. If a graph is plotted of load against time, it is usually found that the curve gradually becomes parallel to the load axis, even though in some cases several hundred hours may be necessary (Wood and Chamberlain, 1954; Abbot and Lowry, 1957; Popplewell and Ward, 1963). This technique can in some cases yield important information regarding the structure of the material, for example the work of Schottelius (1957) on shortened skeletal muscle.

A simple and effective apparatus has been developed for the measurement of "linear plasticity" of leather (Popplewell and Ward, 1963). The specimen was mounted vertically between two jaws, the top one of which was suspended from the centre of a stiff, steel beam which was supported at its ends. The deflection of this beam caused by tension in the specimen was measured by a dial gauge mounted at its centre. In order to achieve a very rapid extension the bottom jaw was fitted to a piston

which moved in a cylinder. Nitrogen gas under high pressure was admitted to the cylinder, causing the piston to move. Full extension could thus be achieved in less than 0.1 seconds. The rapid initial relaxation was recorded by taking a cine film of the dial gauge and a stop watch. Tests were carried out at controlled humidity for relaxation times up to 10^4 minutes.

An interesting and refined piece of apparatus has been built by Wood and Chamberlain (1954). A very rapid extension was produced by a loaded spring, which was released by the action of a trigger and forced the extending jaw against a stop which determined the total degree of extension. So rapid in fact was the extension that it was found necessary to employ a hydraulic damper to prevent the extending mechanism from bouncing when it hit the stop. Load was determined as in the previous apparatus by measuring the deflection of the centre of a steel beam. However, in this case the small displacement of the spring was measured by observing the capacity between the two small parallel plates. This change in capacity was measured electrically. The corresponding variations of tension were displayed on the vertical axis of an oscilloscope with time on the horizontal axis. Indication of time lapse was achieved by superimposing a 50

cycle per second oscillation. This same apparatus was used by Wood (1954) in the investigation of tensile properties of elastic tissue and a similar system was used by Chamberlain and Snowden (1948).

Investigation of stress relaxation of elastin has been carried out (Hall, 1964) using the equipment developed by the present author. A specimen was rapidly extended by revolving the stretching mechanism, a process which could be carried out in one second. The chart mechanism was run with the electric clutch un-energised. A stress-time curve was produced with load on the horizontal axis and time on the vertical axis.

5. Impact testing.

As the name implies, this method involves subjecting the specimen to an impact and observing the resulting deformation or degree of destruction. This is a specialised technique, and is only suitable for certain applications. It yields little fundamental information regarding the structure of the specimen and consequently has rarely been used.

Various forms of impact testing have been evolved. Some of these depend on measuring the amount of energy required to break a specimen. An example of this is the Izod impact testing machine (Low, 1949) in which a pendu-

lum with a heavy mass is dropped from a given height to hit a specimen situated vertically below the fulcrum. The pendulum is then permitted to continue swinging freely, the loss of energy being indicated by the diminished amplitude. This type of apparatus however is only suitable for solid material such as bone.

A system has been used by Tate (1964), working in our department, in which a strip of aponeurosis was mounted between two jaws. The lower jaw fitted firmly to the baseplate of the apparatus and the upper jaw was mounted to one end of a lever with an amplification factor of five. The other end of the lever was fitted with a 20 lb. mass giving a total available force at the specimen of 100 lbs. A proving ring, fitted with a dial gauge, the maximum deflection of which was retained, was mounted in the link between the top jaw and the lever. The test was carried out by releasing the 20 lb. mass. The actual tension required to rupture the specimen was recorded on the dial gauge. This apparatus was developed to investigate the problem of the burst abdomen following surgery. Because it produced the rupture of tissue under sudden loading conditions, the values of maximum tensile stress recorded differed from normal static tensile stress due to plastic effects.

6. Dynamic systems.

In this mode of testing the specimen is subject to high frequency oscillation and the resulting forces and deformations observed. This method is capable of yielding a great deal of information, but the complexity of the apparatus and the mathematical techniques required to process the results are often prohibitive. A typical system of this type consists of a transducer in the form of a dynamic oscillator. This is energised by the tuneable oscillator system and imposes a vibration of fixed amplitude on the specimen. The resulting forces are detected by a sensitive pickup and, after amplification, can be displayed on one axis of an oscilloscope screen, while the displacement is displayed on the other axis. From the resulting figures on the screen the characteristics of the specimen, for example the dynamic Young's modulus, may be calculated (Bergel, 1961b).

Buchthal, Kaiser and Rosenfalk (1949) used this technique to investigate the properties of muscle fibre. Load to the specimen was applied by an electro-magnetic transducer and the displacement was measured by a photoelectric system, to reduce the problem of inertia. The loads and extensions were displayed on the screen of an oscilloscope. The configuration of the resulting

Lissojous figures gave an indication of the properties of the material. This was an ambitious system and produced some very interesting results.

Bergel (1961b) investigated the dynamic response of arteries by subjecting them to an internal hydraulic pressure. This pressure was subject to pulsatile variation, produced by an electric pulsator and the resulting pressure waves recorded by a sensitive pickup. The results were again displayed on the screen of an oscilloscope. Ths visco-elastic properties of the aorta were then investigated at various frequencies.

7. Special and empirical techniques.

Numerous workers have devised specialised techniques to solve their own particular problems, without yielding any fundamental information regarding the properties of material. (Dick, 1949; Hall, 1952). In these cases only comparisons between specimens can be made and no fundamental properties can be calculated, as it is not known in many cases exactly what is being measured.

A novel and original system for testing skin was used by Dick (1949). The specimen was mounted as a circular diaphragm, one side of which was subject to hydrostatic pressure which could be increased uniformly by raising the effective head. The deflection of the centre of the

diaphragm was amplified by a lever and read off a scale. There are certain disadvantages with this type of equipment. The first was that tension in the specimen was calculated from the displacement of the centre of the diaphragm. This was based on the theory for the deflection of diaphragms which only holds for deflection considerably less than those experienced in this case. Thus it is difficult to assess the accuracy of the tension measured. Furthermore, our present work has shown that properties of skin vary in different directions. The relatively large size of specimen required (effective diameter 5 cm.) eliminated the possibility of using biopsy material. The method precluded the determination of the humidity at which the specimen was being tested and this is known to effect results considerably. No facilities were provided for automatic recording of results. Nevertheless, the equipment was used to produce some interesting data.

Various types of fatigue tests have been carried out on fibres, often in the leather industry (Maeser, 1944; Carter and Kangy, 1954). In this type of experiment the specimen is bent backwards and forwards until it ruptures. The total number of cycles which may run into several million is recorded, and gives indication of the durabi-

lity of the material.

An elegant piece of apparatus which is an adaptation of the chain loading system previously mentioned has been used to investigate the energy and entropy effects of collagen fibres (Hall, 1952). A specimen was immersed in water, the temperature of which was varied. The specimen was subject to a load which in spite of the temperature changes was varied in such a way as to maintain a constant length. This was achieved by having the winch of the chain loading mechanism driven by a mag-slip servo system which was controlled by the length of the specimen. This equipment enabled some interesting investigations to be carried out which throw light on the structure of collagen fibres.

An apparatus used by several workers (Kirk and Kvorning, 1949; Kirk, 1950) is known as the Schade elastometer (Schade, 1912). It consists of three small hemispheres arranged in a triangle. These are pressed lightly against the skin of a patient. A fourth hemisphere situated in the centre of the triangle is then pressed against the skin under the action of a known force and the corresponding deflection observed. It is difficult to decide what is actually being measured by this apparatus, as numerous factors will influence the results,

for example skin thickness, skin tension and the condition of the subcutaneous fat. It is for these reasons that the equipment has not enjoyed wider usage.

Clark investigated the effect of hydrostatic pressure on the dural sack and eyeball (Clark, 1932; Clark, Weed and Flexner, 1932; Clark, 1933). The difficulty of the method lies in the lack of uniformity of the surface. Mathematical analysis of the results would be formidable, as it is unlikely that a truly spherical shape would be preserved. Consequently, it is doubtful if any really valuable information can be obtained from this technique.

Jochimes (1943) described a device for measuring the "resiliency" of skin. This system depended on measuring the force necessary to compress the skin of a patient into a fold. A similar system in the form of a spring loaded caliper (Sodeman and Burch, 1938) has also been used. These are both subject to the same criticism as the Schade elastometer and it is difficult to tell what is actually being measured.

A system has been developed by Doerks (1949) in which two parallel lines are drawn at a set distance apart on the surface of the skin of a patient. The two lines are forced together under the action of a light pressure, and their separation measured at the point where a fold

is just about to form. This technique, if carefully used, could possibly give some information as to the tension and extension of skin under normal conditions. Again, however, it is subject to the same difficulties outlined in discussing the Schade elastometer.

An apparatus working on a similar principle to the Schade elastometer has been described by Tui et al (1950). The displacement or depression of skin under the action of given weights gave an indication of response to treatment of scleroderma.

8. Ideal system.

An ideal system would be one which would require the satisfaction of a number of conflicting conditions. It would be simple and capable of producing reproducible, accurate results. It should measure characteristics which could be interpreted in terms of physical properties of the material under conditions which are completely known and reproducible. It is preferable that the results be recorded automatically, so that the errors of human assessment can be eliminated. This can be achieved for example either by an analogue system, where the results can be presented as a stress strain graph for instance (Probine, 1959) or by a digital system where a number of numerical values are printed out.

B. THE COMPONENTS OF SKIN AND THEIR MECHANICAL PROPERTIES

1. THE COMPONENTS OF SKIN

Chemical and histological investigation of skin reveals that it is made up largely of fibres of collagen and elastin (Weinstein and Boucek, 1960). Reticulin is also present in small amounts (Highberger, 1936). These fibres form a meshwork and are surrounded by ground substance.

Collagen.

Collagen is often referred to as white fibre from its microscopic appearance. It is a family of proteins and accounts for approximately 70% of the dry fat free weight of skin. Chemical investigation reveals that it contains numerous amino acids, of which a large proportion (one-third) is glycine (Bowes, 1955). It contains two unusual amino acids hydroxyproline (about 15%) and hydroxylysine (about 2%).

The molecular structure of collagen is found to be very complex and has been studied by x-ray difraction (Rich and Crick, 1958). The generally accepted structure consists of three polypeptide chains coiled together in a helical fashion like a three stranded rope. The three chains are held together by hydrogen bonds between CO and

NH groups of glycine residues in adjacent peptide chains. Every third amino acid in the chain is glycine, but the other third of the amino acid residues consists of proline or hydroxyproline. Glycine has only one hydrogen atom in place of the side chains of the other amino acids, and the sites where it is found form a sort of groove running spirally round the molecule.

The collagen molecule is very large and has a molecular weight of about 340,000 (Hall, 1961). Electron microscopic investigation reveals that these are rigid rods of about 2,800 Å long and about 14 Å in diameter (Schmitt and Hodge, 1960). This molecule is usually referred to as tropocollagen. Microscopic examination of collagen fibrils shows that they have a banded appearance. These cross striations occur regularly at intervals of approximately 640 Å (Curran and Clark, 1963). There is in addition a more pronounced system with a periodicity of 2,400 Å (Hall, 1961). Harkness (1961) refers to this periodicity as occurring at intervals of 2,800 Å units.

It has been shown (Bear, 1952) that the tropocollagen molecules arrange themselves parallel to one another in great numbers to form a larger unit known as a fibril. In the case of human skin these fibrils have been found

to range from 600 to 1100 Å units in diameter (Schmitt, Hall and Jackus, 1942). Large numbers of fibrils are arranged parallel to one another to form an even greater unit known as the fibre. These are several microns in diameter and can be seen with the naked eye. They form the basic meshwork from which skin is built up.

Elastin.

Knowledge of the chemistry of cutaneous elastin is not nearly so advanced as that of collagen. One reason for this is that elastin is not easily available in large amounts in skin, since it only makes up 2-4 per cent of the dry weight (Rothman, 1953; Weinstine and Boucek, 1960).

Elastin fibres are often referred to as yellow fibres, a characteristic which enables them to be distinguished easily from collagen fibres by microscopy. Elastin is made up of a large number of amino acids, of which glycine, alanine, valine and proline form the major part. Histologically elastin appears like closely folded chains (Burton, 1954). The extensibility of elastin has been explained by the fact that the number of polar amino acids is comparatively few (Gross, 1950), hence there must be few cross bonds between lateral molecules and the fibres can be stretched reversibly. The few cross bonds

that are present however must be extremely strongly held, since elastin is insoluble in all the common protein solvents (Lloyd and Garrod, 1946). Microscopically elastin fibres appear to be homogenous, highly contorted and branched; their diameters vary from a few Anstrom units to many hundreds (Rothman, 1953).

Reticulin.

The dermalreticular fibres represent 0.38% of the dry weight of collagen of skin (Highberger, 1936). No amino acid analysis of dermal reticulin has been made, but it is thought to be similar to that of collagen (Bowes and Kenten, 1949). The main differences between reticulin and collagen seems to be in their particular histological staining properties, reticulin taking up silver from silver nitrate solutions, whereas collagen will not (Hall, 1961). There has been considerable discussion about whether in fact reticulin differs from collagen and whether it may be an early stage in the development of collagen.

Ground substance.

The ground substance of connective tissue includes all the non-cellular, non-fibrous material which is present in the tissue (Well, 1953). To the histologist it appears to be a protein and polysaccharide bearing mass

lying outside the fibres (Hall, 1961).

2. THE MECHANICAL PROPERTIES OF THE FIBROUS COMPONENTS OF SKIN.

The usual mechanical properties which are used to describe the characteristics of the material are as follows:-

1) Tensile strength. This is the force required to rupture the specimen in tension and is given in units of load per unit area.

2) Extensibility or elongation. This is the increase in length of the specimen at rupture given as a percentage of the original length of the specimen.

3) Creep. This is a phenomenon observed when a specimen continues to extend under the action of a constant force.

4) Stress relaxation. This phenomenon is observed when the specimen is suddenly deformed and the load resulting from this deformation is found to decrease with time.

5) Young's modulus. This is defined as the gradient of the stress strain graph, and is of great importance in certain materials such as steel, where the relationship is linear. However in cases where stress and strain have a non-linear relationship to one another it is

of little use since it is difficult to decide at what portion of the curve to take the gradient.

COLLAGEN.

The mechanical properties of collagen are dependent on the relative humidity at which the specimens are tested (Morgan, 1960). The tensile strength is at maximum when the water content is between 10 and 20% (Mitton, 1945). The pH and the temperature of the medium surrounding the specimen also affects the properties (Hall, 1951).

a) Tensile strength.

It is difficult to compare values obtained by various workers as experimental conditions differed in most cases and consequently a wide range of values (between 10 and 50kg. per sq. mm.) have been reported. With dermal collagen it was found that the shorter the specimen the higher the tensile strength (Mitton and Morgan, 1960). This is probably because there is a higher proportion of fibre components running at angles to the direction of stress in larger blocks of tissue (Mitton, 1945). Highberger (1947) suggested that rupture does not involve breaking of peptide bonds but rather slip between or within the fibrils. A value of 49 kg. per sq.mm. has been reported for single bovine dermal collagen fibres by

Sleighsberger, Mann and Clayton (1960), while a figure of 13 kg. per sq. mm. has been found for human tendon (Cronkite, 1936), though this would appear to be low compared with the value of 50 kg. per sq. mm. for tendon collagen given by Hall and Jackus (1942). A value of 5 kg. per sq.mm. for human fascia lata was found by Gratz (1931), referring to tissue rather than single fibres. Bone was found to have a value of tensile strength of 13 kg. per sq. mm. (Elftman, 1949). Some workers have found the tensile strength of collagen by testing pieces of collagenous tissue and estimating the value from the proportion of collagen that the specimens contain. A value of 10 kg. per sq. mm. has been found for human dermal collagen, assuming that collagen is 20% of the wet weight (Rollhauser, 1950b). By similar means the value for frog skeletal muscle has been found to range between 3 and 5 kg. per sq. mm. (Walter, 1947; Casella, 1950). For rat cervix uteri a value of between 3 and 5 kg. per sq. mm. has been reported (Harkness and Harkness, 1959a). The wide variation between these results is due partly to differing experimental techniques. It is also of interest to note that even when subject to tanning processes the tensile strength of collagenous tissue is only slightly reduced (Mao and Roddy, 1950).

b) Extensibility.

The extensibility of collagen is usually relatively low and falls between 10 and 20%, although under special conditions considerably greater extensions have been observed (Schmitt, Hall and Jackus, 1942). Harkness and Harkness (1960) noted the large extensions of the cervix of the rat during pregnancy, and suggested that this may partly be due to slip of the micro-fibrils over one another, possibly due to some enzymatic activity. Morgan (1960) showed that maximum extension of native collagen fibres depended on relative humidity, and was apparently maximum at 66% relative humidity. Completely dry fibres were brittle and gave a low value. Microscopic examination of single fibres during extensions showed that the 640 Å band spacing increased proportionally (Cowen, North and Randall, 1955). With single collagen fibres the incremental increase in extension was found to diminish as the load increased (Morgan, 1960). This phenomenon has been observed by numerous other workers (Hall, 1951; Hill, 1951; Wood, 1954). The initial part of the extension was reversible and this was thought to be due to an orientating effect.

Much higher values have been reported for the extensibility of collagenous tissues. Rothman (1953)

gave a value of nearly 50% for juvenile skin and our present work has indicated that values of over 100% can occur in certain conditions. These large values obtained with tissue must to a considerable extent be due to the orientating of the collagen fibres within the tissue. This is born out in our work by the fact that the extensibility of a piece of skin is dependent on the direction in which a specimen is cut, although obviously properties of the collagen will not change.

c) Young's modulus.

Comparisons between moduli have little meaning without some definition of the region of the stress strain curve from which they have been obtained. It seems probable for example that some of the reported changes in elasticity of tissue with age have been due to different parts of the curve being used (Harkness, 1961) and values ranging from 10 to 1000 gm. per sq. mm. have been reported by various workers (Hirsch, 1944).

d) Plastic properties.

There are few reports about plastic properties of collagen. However Hall (1951a) found that wet dermal bovine fibres under the action of a constant load reached a point where they extended at a mere 0.03% per hour after a period of several days.

ELASTIN

a) Tensile strength.

The tensile strength of elastin is considerably less than that of collagen. A value of 0.1 kg. per sq. mm. for elastin taken from the aorta was found by Burton (1954) and an even lower value of 0.0675 kg. per sq.mm. has been reported (Hass, 1942; Krafka, 1942).

b) Extensibility.

One of the main characteristic properties of elastin is its large extensibility. Values of 100% are common (Wohlisch, 1943; Burton, 1954).

c) Young's modulus.

Hall (1964) using the apparatus described in this communication has investigated thin sheets of ligamentum nuchae. He found that stress bore a perfectly linear relationship to strain over a considerable range. This was important in that Young's modulus could be accurately determined. In general there is good agreement between the results obtained by the workers in this field. A value of 3×10^6 dynes per sq. cm. was quoted by Harkness (1957) for elastin contained in the arterial wall of the dog. A similar value was quoted by Burton (1954) for walls of blood vessels. The same value was obtained for ligamentum nuchae (Krafka, 1937). However, a value

of less than one tenth of this was found for aorta by the same worker.

d) Plastic properties.

Hall (1964) showed that if a specimen of elastin from ligamentum nuchae was extended at constant rate, on removal of the load it returned to its original length, and on re-testing an identical stress strain curve was produced. This confirmed the result of Krafka (1939), who also worked with ligamentum nuchae. Elastin therefore exhibits no creep or stress relaxation.

C. THE MECHANICAL PROPERTIES OF SKIN.

There are surprisingly few references to work on mechanical properties of skin. The most significant work in this field has been done by Wohlisch(1927), Jochimes (1943), Wenzel (1949), Rollhauser (1950), Dick (1951), Kenedi, Gibson and Daly (1963) and Kenedi (1964). It is of interest that most of the work has been carried on outside Great Britain, there having been apparently little interest in the subject until recently in this country. The properties which have been described will be discussed under separate headings.

1. Tensile strength.

Tensile strength has been most frequently investigated. It has been found to vary with age, sex, the site and direction from which the specimen has been taken, and various disease states. The most complete study of the variation of the tensile strength with age has been carried out by Rollhauser (1950), who stated that in infants up to three months of age the value was only 0.25 to 0.3kg. per sq. mm. In children 3 months to 3 years old, it was 0.53 to 1.4kg. per sq. mm. In adults from 15 to 50 years of age the average value was 1.61 kg. per sq. mm. and in persons from 50 to 80 years old 2.05 kg.

per sq. mm. An average tensile strength of 1.8 kg. per sq. mm. has been quoted by Wohlisch (1927). Much work on the properties of skin was carried out by Wenzel (1949). His results are qualitative rather than quantitative. He reported that tensile strength was very dependent on the rate of loading, rapid loading giving a value many times that of the static loading. He also stated that the tensile strength of female skin was less than that of male. With pregnancy the tensile strength was even lower, being similar to that of a baby. The effect of certain diseases, such as Cushing's syndrome was to render the skin similar to that of a baby or girl. He noted that the strength was greater in the direction of Langer's lines than it was at right angles to them.

It has also been found (Rollhauser, 1950) that abnormally low values of tensile strength were associated with patients who died from "consumptive disease" (presumably tuberculosis). However the lowest values of all have been reported for a case of Ehlers Danlos syndrome where a value for a 35 year old man was 0.34kg. per sq. mm. Rollhauser also suggested that the tensile strength could be due partly to its water content, and postulated that the lower values obtained for juveniles may be due in part to this. Indeed Dick (1949) showed

that the properties of oedematous skin differed widely from those of normal, but quoted no actual values of tensile strength. Rollhauser also noted that differences in tensile strength of skin between individuals were closely paralleled by similar differences in tensile strength of tendons, suggesting that the properties of collagen throughout the body varied similarly. This finding was also endorsed by Wenzel (1949). Wohlisch (1927) observed that the tensile strength of epidermis plus dermis was greater than the tensile strength of dermis alone. This suggests that in spite of its thinness the epidermis has appreciable tensile strength, although compared with the total tensile strength it is negligible.

2. Distensibility.

The distensibility of skin decreases with increasing age. Rollhauser (1950) quoted values of extension of 50% to 59% for strips of skin taken from infants at birth. For children it varies between 37% and 52% and for adults 24% to 48%. This finding was born out by the work of Dick (1949). Work carried out on the Schade elastometer (Schade, 1912; Kirk and Kvoning, 1949; Kirk, 1950) showed that the "elastic" behaviour of skin varied

with age. However, as previously mentioned, the results from this apparatus could be due in part to factors other than the properties of skin. Various other workers have carried out in vivo tests (Jochimes, 1934; Doercks, 1949; Tui, 1950; Olmsted, 1951) but these results are subject to the same criticism as those from the Schade elastometer.

Abnormal cutaneous distensibility has been observed clinically in patients suffering from Ehlers Danlos syndrome (Job van Meekeren, 1657; Jansen, 1954; Ellis and Bundick, 1956; McKusick, 1956; Robinson and Ellis, 1958) but quantitative observations are sadly lacking for this disease.

3. Young's modulus.

It is difficult to quote a value of Young's modulus for skin since, in common with many other biological tissues, the stress strain curve is not linear. The general form of the curve for various biological tissues is well established, commencing tangential to the extension axis, gradually bending towards the load axis (Werthheim, 1841; Wundt, 1856; Roy, 1880; Dick, 1949; Wenzel, 1949; Rollhauser, 1950; Hall, 1951; Morgan, 1960; Harkness, 1961; Kenedi, 1964). With a curve form of this

type it is imperative that the load at which the modulus is calculated be quoted. This however has not been done by many workers and their results are difficult to compare.

4. Non-specific properties.

Numerous workers have observed that skin of the normal individual is permanently subject to a slight tension (Evans, Cowdry and Nielson, 1943; Dick, 1949; Wenzel, 1949). This tension, it is generally agreed, becomes less with advancing age. It was found to be greater in one direction than at right angles to it (Langer, 1861). Wenzel (1949) investigated the shrinkage of pieces of exised skin due to the relaxation of this tension and found that it was greater in men than women, the respective values being 14% and 10.7%. He also found that these reduced respectively to 6.3% and 8.2% when taken at right angles to the direction of principle tension.

Wenzel also reported that a piece of skin could be apparently "aged" by mechanical conditioning. The precise mechanism of this is not understood, though it is probably due to some fatigueing process acting on the wickerwork or cross links of the structure. On the other hand Goldzieher (1949) and Chieffie (1950), stated that by the application of massive doses of hormonal

preparations, such as estrogen and androgen a regenerative process may take place, particularly in senile women, and produce "a more youthful type" of stress strain curve.

D. THEORETICAL APPROACHES TO THE STRUCTURE OF COLLAGEN

Numerous theoretical approaches have been derived to explain the behaviour of elastomers, most of which originated in the rubber industry, and have been applied by some workers to the field of biology (Hall, 1951; Trelaor, 1958). A few of the more popular systems have been discussed below.

1. Kinetic theory of elasticity.

Wall (1942 a & b) attempted to explain the behaviour of rubber by considering the entropy of the whole system, and by using statistical methods arrived at the equation:-

$$F = \frac{NkT}{l_o}\left[\frac{l}{l_o} - \left(\frac{l_o}{l}\right)^2\right]$$

where F = force
N = total number of molecules
k = Boltzman's constant
T = absolute temperature
l_o = initial length
l = extended length

Unfortunately this method may be limited to rubber on account of its large extensibility, and cannot be expected to apply for all polymeric substances, since no account has been taken of intermolecular forces, or deformation other than free rotation effects. Hall (1951) found this analysis inadequate to describe collagen,

where elongation at rupture is only of the order of 25% as opposed to about 700% for rubber.

Much work has been done (Meyer, Susich and Valko, 1932; Guth and Mark, 1934; Kuhn, 1934 and 1936; Pelzer, 1938) by applying reasoning similar to the above at the molecular level. This analysis has been further developed by Frenkel (1946) who produced the following equation

$$F = kT\left[\frac{3\,l}{a^2 Z} - \frac{2}{l}\right]$$

where F = force
a = length of chain link
Z = number of links
l = length of chain

Hall (1951b) developed this expansion:-

For a bundle of n molecular chains, we have

$$F_o = nkT\left[\frac{3l_o}{a^2 Z} - \frac{2}{l_o}\right]$$

Where l_o is length of chain when extended by a force F_o and

$$F_1 = nkT\left[\frac{3l}{a^2 Z} - \frac{2}{l_1}\right]$$

Where l_1 is length of chain when extended by a force F_1 hence

$$F_1 - F_o = nkT\left[\frac{3}{a^2 Z}(l_1 - l_o) - \left(\frac{2}{l_1} - \frac{2}{l_o}\right)\right]$$

Let $F_o = 0$ then $l_1 - l_o = l_e$ the extension produced by a force F_1 therefore

$$F_1 = nkT\left[\frac{3}{a^2 Z}\, l_e + \frac{2l_o}{l_o(l_o+l_e)}\right] \quad \text{.....(3)}$$

Schmitt (1944) found that collagen fibrils showed alternate light and dark bands when examined under the electron microscope, and that on stretching the light bands extended at the expense of the dark ones. Hence it does not follow that l_o in the above equation will be equal to the total length of the unstretched fibre. Let $l_o = L$ a constant to be determined.

The equation now reduces to:-

$$F + a_1 l_e + a_2 l_e^2 + a_3 F l_e = 0 \quad \text{.....(4)}$$

Where $a_1 = -nkT\left[\frac{3}{a^2 Z} + \frac{2}{c^2}\right]$

$$a_2 = -\frac{3nkT}{a^2 ZC}$$

$$a_3 = \frac{1}{C}$$

Hall found it possible to fit this equation by the method of least squares to the curve obtained from collagen fibres tested over a wide range of pH. The constants a_1 a_2 and a_3 were calculated, but it is difficult to interpret the fact that a_3 was found to have a negative sign. For this reason it is seen that this

approach is not completely adequate to describe the behaviour of collagen.

2. Thermodynamic analysis.

The stress required to extend a rubber-like substance can be divided into internal energy and entropy components (Wiegand and Snyder, 1934; Frenkel, 1946; Treloar, 1958).

Let T = absolute temperature
F = force to extend fibre
l = length of fibre
Q = heat absorbed
E = internal energy
W = work done by material = Fdl

ϕ = entropy: $d\phi = \frac{dQ}{T}$

From the first law of thermodynamics

$$dQ = dE + dW \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

And from the second law

$$Td\phi = dE + dW$$
$$= dE - Fdl \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Therefore at constant temperature

$$F = -T\left(\frac{\partial \phi}{\partial l}\right)_T + \left(\frac{\partial E}{\partial l}\right)_T \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Differentiating (3) with respect to T we get

$$\left(\frac{\partial F}{\partial T}\right)_l = -T\left(\frac{\partial^2 \phi}{\partial T \partial l}\right) - \left(\frac{\partial \phi}{\partial l}\right)_T + \left(\frac{\partial^2 E}{\partial T \partial l}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Similarly, for constant length l from (2)

$$\left(\partial \phi\right)_l = \left(\frac{\partial E}{T}\right)_l \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$\frac{\partial \phi}{\partial T} = \frac{1}{T}\left(\frac{\partial E}{\partial T}\right)_l \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Differentiating with respect to l we get

$$\left(\frac{\partial^2 \phi}{\partial T \partial l}\right) = \frac{1}{T}\left(\frac{\partial^2 E}{\partial T \partial l}\right) \qquad \ldots\ldots\ldots\ldots\ldots(7)$$

Combining (7) and (4)

$$\left(\frac{\partial F}{\partial T}\right)_l = -\left(\frac{\partial \phi}{\partial l}\right)_T \qquad \ldots\ldots\ldots\ldots\ldots(8)$$

i.e. Maxwell's identity

combining (8) and (3)

$$\left(\frac{\partial \Xi}{\partial l}\right)_T = F - T\left(\frac{\partial F}{\partial T}\right) \qquad \ldots\ldots\ldots\ldots\ldots(9)$$

The problem of work done by the change of volume of the specimen in a hydrostatic pressure has been investigated by several workers. Elliott and Lippmann (1945) introduced an additional term to equation (9) which became

$$\left(\frac{\partial E}{\partial l}\right)_{V,T} = F - T\left(\frac{\partial F}{\partial T}\right)_{l,P} - M\beta T A \qquad \ldots\ldots(10)$$

where M = extension modulus
V = volume of specimen
P = hydrostatic pressure
p = thermal coefficient of linear expansion at constant deformation at zero pressure
A = anisotropy factor

$$= \frac{3V}{l}\left(\frac{\partial l}{\partial V}\right)_{F,T}$$

for isotropic material

However Hall (1952) found this additional term to be very small, i.e. 10^{-3} for collagen fibres and therefore neglected it. Treloar (1958) stated this was less than F 1 by a factor of 10^{-3} -10^{-4} for rubber.

King and Lawton (1950) introduced a term -PdV into equation (1) as part of the work term, but it was again found that this could be neglected for rubber-like materials, some of which exhibit isovolumetric extensions of up to 300 per cent (Holt and McPherson, 1936).

Equation (9) while being a description of the process of extension is not in its most convenient form.

Multiplying (8) by T we have

$$T\left(\frac{\partial F}{\partial T}\right)_l = -T\left(\frac{\partial \phi}{\partial l}\right)_T = -\left(\frac{\partial Q}{\partial l}\right) \quad \ldots \quad (11)$$

Substituting in (9)

$$\left(\frac{\partial E}{\partial l}\right)_T = F\left(\frac{\partial Q}{\partial l}\right)_T \quad \ldots \quad (12)$$

Integrating (12) for a change of extension between l and l

$$\int_{l_2}^{l_1}\left(\frac{\partial E}{\partial l}\right)_T dl = \int_{l_2}^{l_1} F\,dl - \int_{l_2}^{l_1}\left(\frac{\partial Q}{\partial l}\right) dl \quad \ldots \quad (13)$$

That is, we have an expression giving the total work done during extension $\int_{l_2}^{l_1} F\,dl$ in terms of the sum of the changes of internal energy and entropy. (The term $\frac{\partial Q}{\partial l}$ is a more convenient expression than $T\left(\frac{\partial \phi}{\partial l}\right)$ which

is equal to it).

Hall (1952) successfully applied this method to collagen fibres in water which had previously been acetone-dehydrated and suggested that the components in equation (13) are in the ratio of 3:2.

3. A developed statistical method.

An analysis, which is a development of a statistical method but for smaller extensibility, has been evolved for systems involving chains with independent links, which it is thought, may be applicable to the structure of collagen. This employed the inverse Langevin function (Guth and James, 1943; Hall, 1951).

This may be written in the form:-

$$F=\frac{1}{3} Ba_o\left\{L^{-1}\left(\frac{a}{a_o}\right) - a^{-\frac{3}{2}} L^{-1}\left(\frac{a^{-\frac{1}{2}}}{a_o}\right)\right\}$$

where F = force
B = constant
A = extension ratio $\frac{l}{l_o}$
a_o = limits extens.
L^{-1} = the inverse Langevin function defined by

$$L(X) = \coth X - \frac{1}{X} = Y$$

$$L^{-1}(y) = X$$

However, it is a difficult problem to fit such an expression to a curve, and it has been shown that this does not

completely describe the curve obtained for skin (Kenedi, Gibson and Daly, 1963).

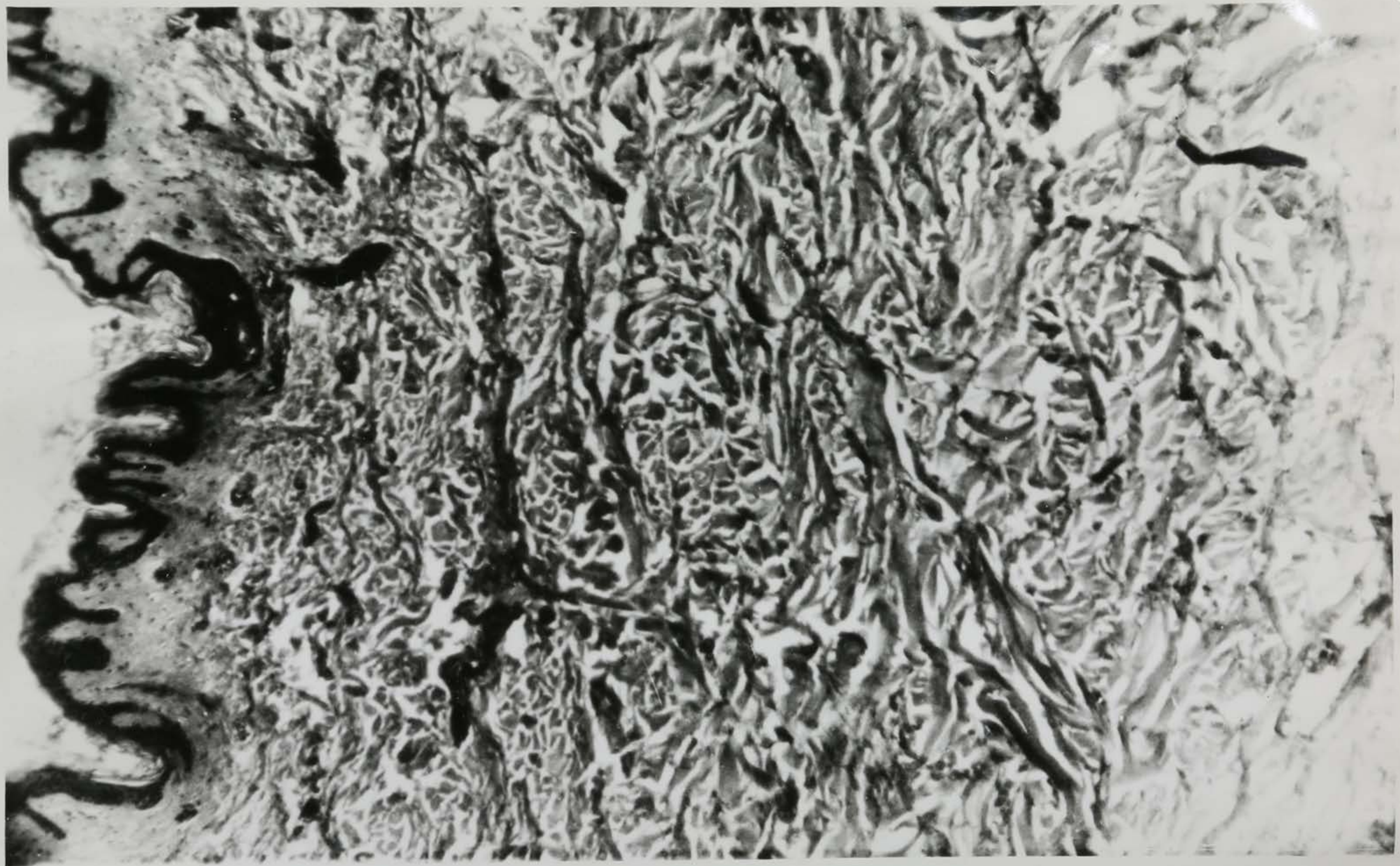
111. STRUCTURE AND PHYSIOLOGY OF SKIN

Human skin consists of two distinct layers, epidermis and dermis (plate 1). The epidermis is external and subject to the adverse conditions of everyday life. It is much thinner than the dermis. Its thickness varies considerably with the site from which the specimen is taken and age and sex of the donor (McCleod and Muende, 1946). These two layers are sub-divided further.

1. EPIDERMIS.

Five separate layers have been described in the epidermis though some of these are ill-defined and there is the gradual transition from one form of cells to another. From the external surface inwards these layers are, stratum corneum (horny layer), stratum lucidum, stratum granulosum (granular layer), stratum Malphighii (prickle-cell layer), stratum germinativum (basal layer). The stratum corneum varies in thickness from 0.02mm. on the flexor surfaces of the forearm to 6.0mm. on the soles of the feet. It forms an effective protective barrier against mechanical and chemical injury and undue absorption or loss of water. Below this the stratum lucidum is seen in section as a semi-transparent line, due to the presence of eleidin. The layer is best seen in the soles and palms. The cells of the stratum granulosum are diamond

PLATE 1. Histological section of skin stained with Mallory trichrome stain (magnification X250).



shaped, and filled with granules, which are coarse, irregular in size and shape, and strongly light refractile. The stratum Malphighii comprises squamous or prickle cells, which are polygonal and form a mosaic, becoming flattened towards the surface of the layer, and spaces traversed by intercellular bridges or prickles, enabling lymphatic fluid to circulate between the cells (Lever, 1949). The deepest layer of the epidermis is the stratum germinativum and consists of columnar cells with their axes normal to the dermal interface. At their bases these cells project downward in the form of the tufts which are embedded in the corium.

There are epidermal appendages, which although associated with the epidermis penetrate into the dermis. These are the sweat glands and hair follicles. The epidermal portion of the duct delivers sweat to the surface of the skin, and in the dermis is situated the gland part of the sweat or ecrine gland and a portion of the duct. The hair follicle passes through the epidermis deeply into the dermis. Sebaceous glands are present over the surface of the body with the exception of the palms and soles. From one to six sebaceous glands are grouped into a pilo-sebaceous follicle, which may or may not contain a hair. The gland consists of a number of fatty cells,

which exude an oily secretion, sebum.

DERMIS (corium).

Below the epidermis is a much thicker layer of dense fibrous tissue, the dermis, which is responsible for the strength and elasticity of the skin. It is this layer which supports and protects the various cells and vessels associated with the skin.

The dermis is normally considered to consist of two layers (McCleod and Muende, 1946), the papillary layer and the deeper reticular layer which merges gradually with the subcutaneous tissue.

Papillary layer.

The papillary layer consists of a loose structure of collagen fibres and forms the bed for the epidermis, there being no separating layer between it and the basal layer. This junction forms the site of many pathological conditions, as both parts are served by a common nutrient and lymphatic system.

The papillary layer is so called because of the presence of papillae - conical projections which contain terminal capillary loops and certain nerve endings, giving rise to the notation vascular and sensory papillae. The density and size of the papillae varies from site to site, being greatest in the soles and palms. It has been

estimated that there are as many as 100 per square millimetres of body surface (Ormsby and Montgomery, 1943). One or more papillae may spring from a common base, which is a bulbous projection into the reticular layer, a rete peg. The papillae are arranged in linear series or concentric whorls in certain parts of the body, and in some places the effect is so pronounced that it becomes visible to the naked eye, and forms the characteristic patterns of finger prints.

Reticular layer.

The reticular layer consists of a tight meshwork of large bundles of collagen fibres arranged with their axes horizontal, there being a preponderance in the direction of Langer's lines. There is also a small number of elastic fibres which form a second meshwork within the collagenous system. The interstices between the fibres, as in the papillary layer, are filled with a semigel/semisol mucopolysacharide compound, known as ground substance (Rothman, 1953).

Muscle.

Skin contains muscles the most common of which are the erector pili muscles which are associated with the hair follicle. The function is to cause the hair to stand erect and also to cause the sebaceous glands to

express their contents. They are of the non-striped type and terminate high in the dermis close to the epidermis.

Blood and lymph vessels.

The skin is provided with a vascular system for both blood and lymph. The larger vessels are found deep in the dermis and radiate upwards in the form of branching capillaries which serve the various glands and cells.

3. LANGER'S LINES.

It has been found that if the skin is pierced in situ with a sharpely pointed instrument the result is an eliptical hole rather than a circular one. This must be due to the fact that the tension in the skin is not the same in all directions. The major axis of the elipse corresponds to the direction of maximum tension and the minor axis to that of minimum tension.

The first observation of the disparity between the shape of an instrument and the shape of the skin wound produced by it was made by Dupuytren (1834). On 20th August, 1831, he was called to treat an attempted suicide at the Hotel Dieu in Paris. This attempt had been made with a stilletto and had resulted in the production of three small precordial wounds. These wounds Dupuytren observed were linear not circular in shape. He was in some doubt whether to believe the assertions of his

patient that the wounds were produced by a round bladed stilletto, or his own observations which strongly suggested an instrument which had a linear cutting edge.

He therefore performed experiments on a cadaver and demonstrated that wounds made in the skin with a conical instrument were linear not circular in shape, and that the direction of these wounds differed in different parts of the body.

If many such holes are pierced in the skin over the whole surface area of the cadaver and the major axis of the elipses joined up, a series of lines will be formed. These are known as Langer's lines (Langer, 1861). They are sometimes referred to as lines of cleavage, and give the direction of principle tension over the surface of the body. The general pattern of Langer's lines are shown in figures 2 and 3 (modified from Foman, 1939).

Langer's lines are important in surgery as they form the preferred direction of incision. The sides of an incision made along the Langer's lines will be pulled together, thus tending to heal more readily, resulting in minimal scarring. Conversely, incisions made across Langer's lines, tend to be pulled apart and heal less readily producing bad scars.

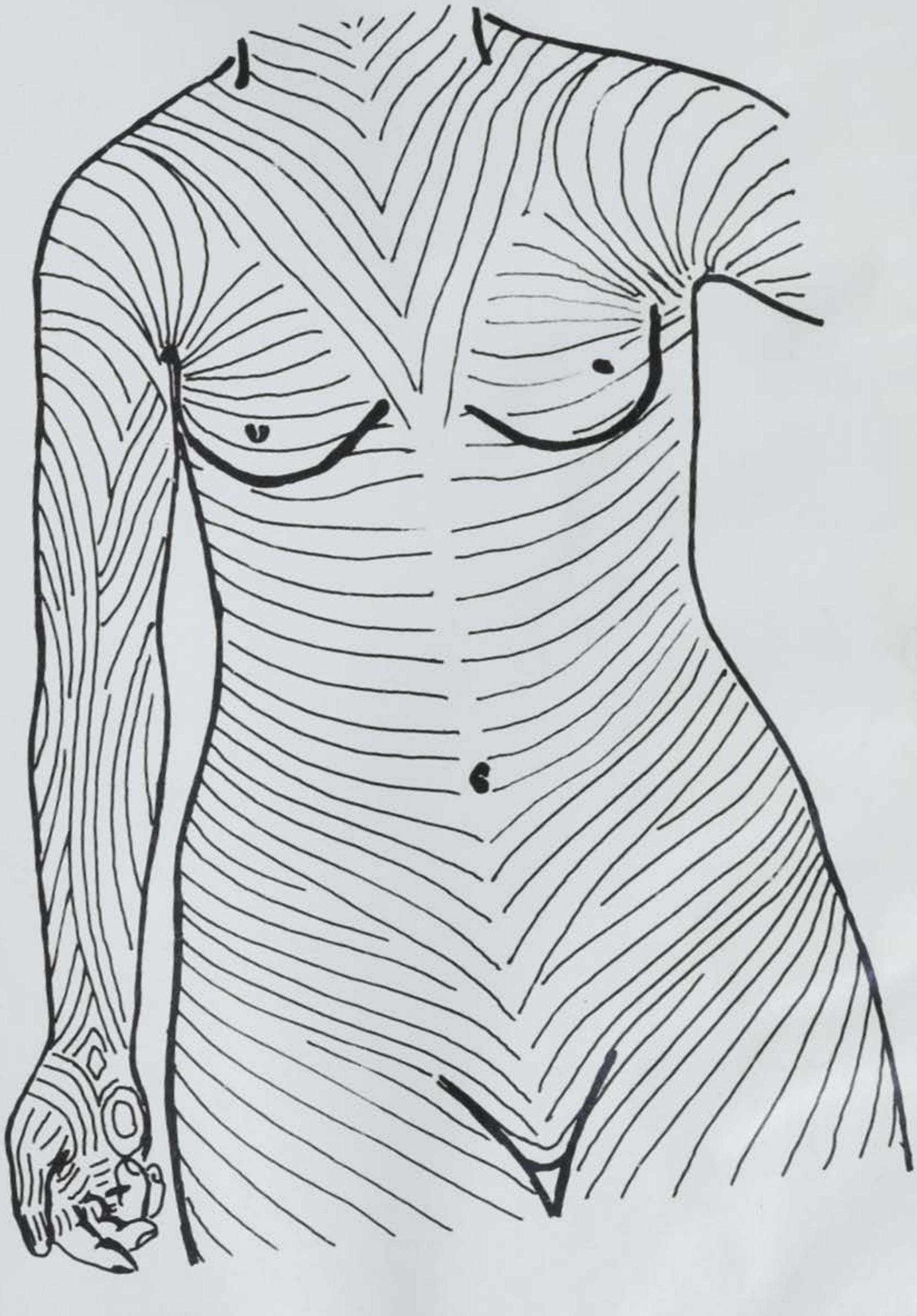


FIG. 2

DIRECTION OF LANGER'S LINES
(modified from FOMAN, 1939)

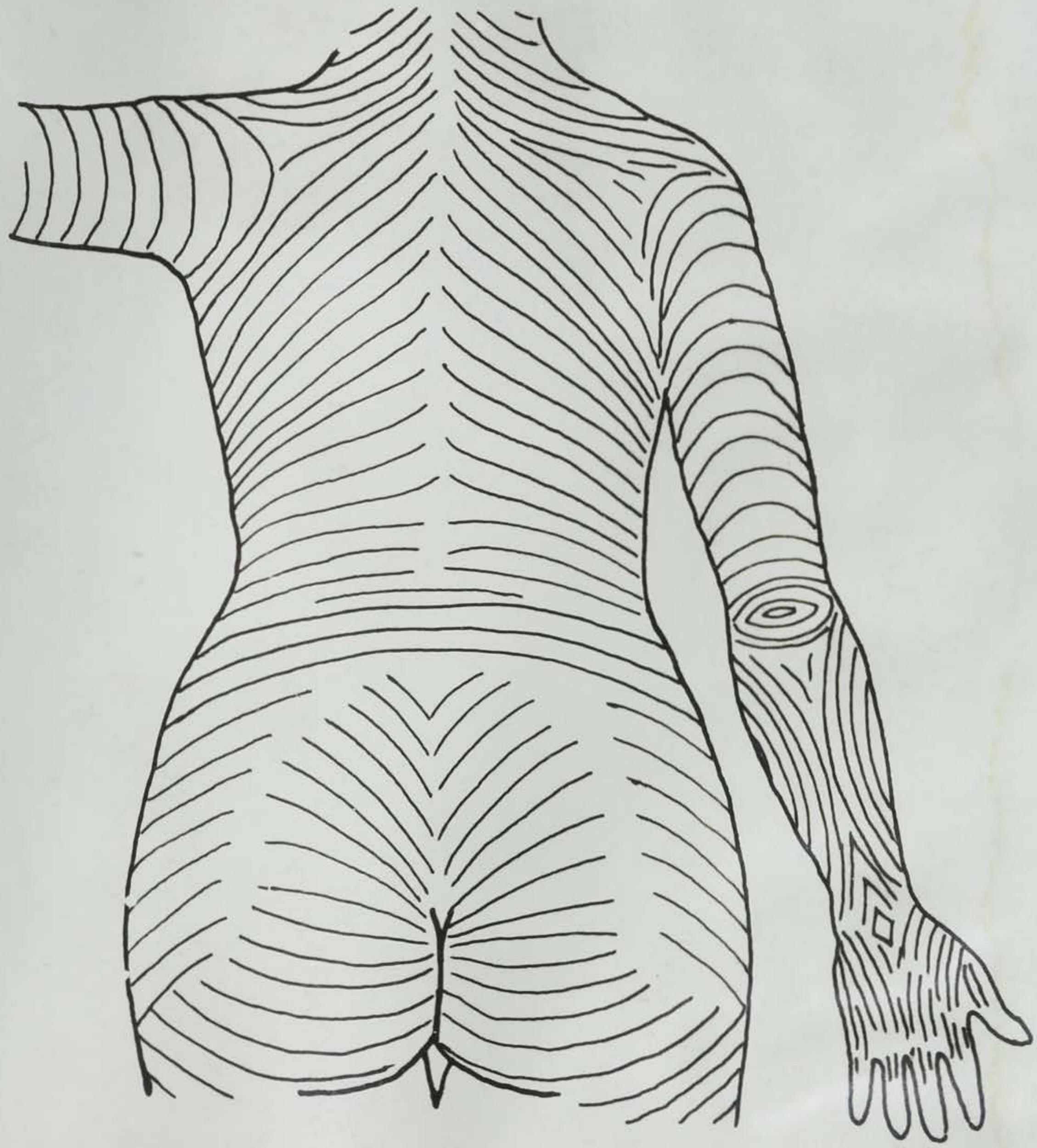


FIG. 3

DIRECTION OF LANGER'S LINES
(modified from FOMAN, 1939)

Kocher (1892) was the first surgeon to recognise the surgical importance of Langer's lines, and as a result of his work the Langer's lines in certain parts of the body are sometimes referred to as Kocher's lines.

Langer's lines lie in the direction of minimum extensibility and perpendicular to the direction in which the skin may be stretched most (Ormsby and Montgomery, 1943). It has also been shown by Cox (1942), who carried out many investigations to establish accurately the direction of the lines, that if histological sections were taken both in the direction of and perpendicular to the lines, the fibres tended to be orientated in the direction of Langer's lines. This accounts for the fact that skin is much less extensible when extended along the lines than across them.

Cox also stated that Langer's lines coincided with crease lines, which are visible on the surface of the skin in most areas of the body. Exceptions to this coincidence of Langer's lines and crease lines were found in the palms, the soles, the flexor aspects of the knee and elbow, and the extensor aspects of the ankle.

Cox also stated that Langer's lines may be demonstrated on a piece of skin which had been excised from the body. This clearly showed that the lines were a property of the skin itself and not the underlying tissue or the

surrounding skin structure.

IV. MATERIAL AND METHODS

A. PREPARATION OF SKIN FOR EXPERIMENT

1. METHODS OF OBTAINING SKIN SAMPLES

Both autopsy and biopsy specimens were used. The techniques for obtaining this material are described below.

Autopsy specimens.

Samples of full thickness human skin were taken from the abdomen, back, forearm and thigh. The specimens from the abdomen were taken from a paramedian position in the epigastric region. Those from the back were taken from a similar position dorsally. Those from the forearm were taken from the volar aspect in the lateral part of the left limb. The upper limit of the incision was two inches below the medial epicondyle of the humerus. Those from the thigh were taken from the left leg on the lateral aspect, the upper border of the specimen being one inch below the greater trochanter of the femur.

Great care was taken in dissecting the specimen to ensure that it was not stretched or deformed. An incision with a sharp scalpel was made around the site, producing a specimen larger than that required for experimentation. The specimen was freed on its undersurface by sharp dissection, leaving a layer of subcutaneous fat on the skin. It was never held under tension to facilitate removal.

The direction of the sample in relation to the body surface was noted, so that it could be positioned with respect to Langer's lines. The specimens were obtained at autopsy within 24 hours of death, and prepared for testing the same day.

Biopsy specimens.

Specimens were taken from the forearm of patients by Dr. V. Wright. The position was the same as that for the autopsy material. The area was anaesthetised by infiltration of 2% procaine around the site from which the skin was to be taken. An eliptical incision was made slightly wider and longer than the dimensions of the specimen required. A full thickness skin biopsy was obtained, the same precautions against stretching being observed as with autopsy specimens. The wound was closed with three mattress sutures to prevent bleeding and superficial skin sutures as needed. One limitation of biopsy material is that the sample obtained is sufficient for only one test. Biopsy specimens of skin were also obtained during abdominal operations. This source, while proving very useful, had the disadvantage that specimens were taken from different sites according to the nature of the operation and so comparison of results was difficult, and the use of such material was finally abandoned.

2. CUTTING OF SPECIMENS

To enable a rheological analysis of the properties of skin to be carried out, it was necessary to be able to cut the skin into strips of uniform cross sectional area, and also maintain a fair degree of reproducibility between any two individual samples.

Preliminary experiments were carried out to investigate the cutting properties of skin, and to get an approximate idea of the stress strain relationships. Autopsy specimens were used at this stage, and a size of 4 x 2 x 250mm. was chosen, since this was the optimum size from a biopsy point of view.

Parallel blade cutter.

Difficulties in cutting specimens of skin arose from the toughness of the epidermis and the plasticity of the dermis, which was greatly deformed as soon as any cutting was attempted.

No accuracy could be achieved by cutting with a single blade, and a cutting die was produced of the design shown in figure 4c, having two parallel blades of hardened high-carbon steel mounted in a mild steel block, to give high rigidity under load. The skin was placed on a hard wooden surface and strips of accurate width were cut by pressing the cutter into it. After carrying out tests

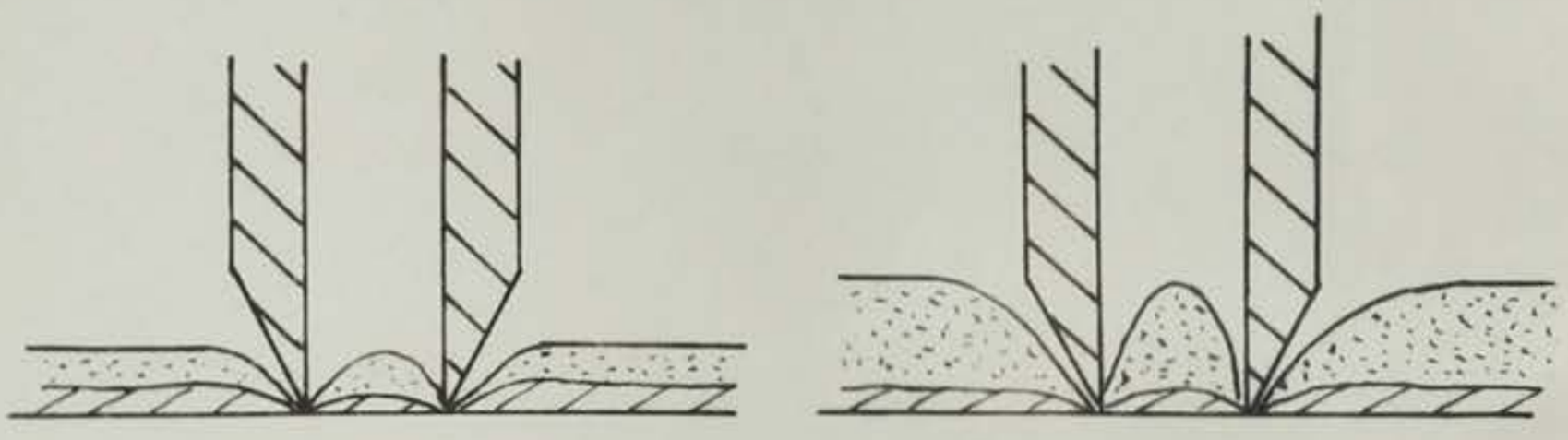


FIG. 4a

FIG. 4b

SPECIMEN SECTION FOR THICK AND THIN SKIN

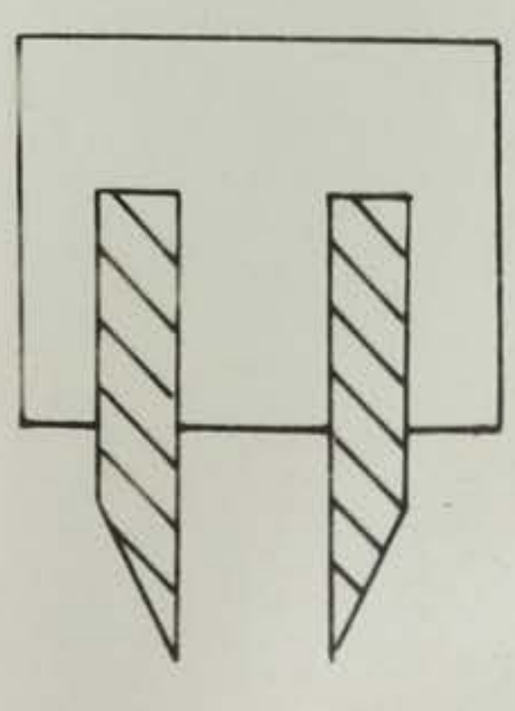


FIG. 4c

PARALLEL BLADE CUTTER

on skins of various thicknesses, it was noticed that the shape of the cross section varied according to the thickness, due to the fact that the thicker the skin the more it was deformed before it was compressed sufficiently for the cutting action to take place. Figures 4a and b illustrate how the thinner samples of skin were not deformed as much as the thicker ones. The section of the strip thus formed was not a perfect rectangle, due to the fact that it had been distorted in cutting.

For the proposed tests uniformity of cross-sectional area was required rather than a particular configuration. All specimens were therefore cut from skins which had previously been reduced to a standard thickness and the problem of different distortion with varying thickness was then obviated.

When testing parallel specimens, it was found that it was not possible to ensure that there was exactly the same length between the jaws (i.e. the 'gauge' length) at each experiment. It was necessary to apply a correlation to standardise the gauge length at 1cm. and so obtain comparable results. Standardisation formulae given in appendix 1.

The parallel cutter was suitable for cutting the skin, but introduced difficulties in testing the specimen. On mounting the test-piece in the jaws of the extensometer,

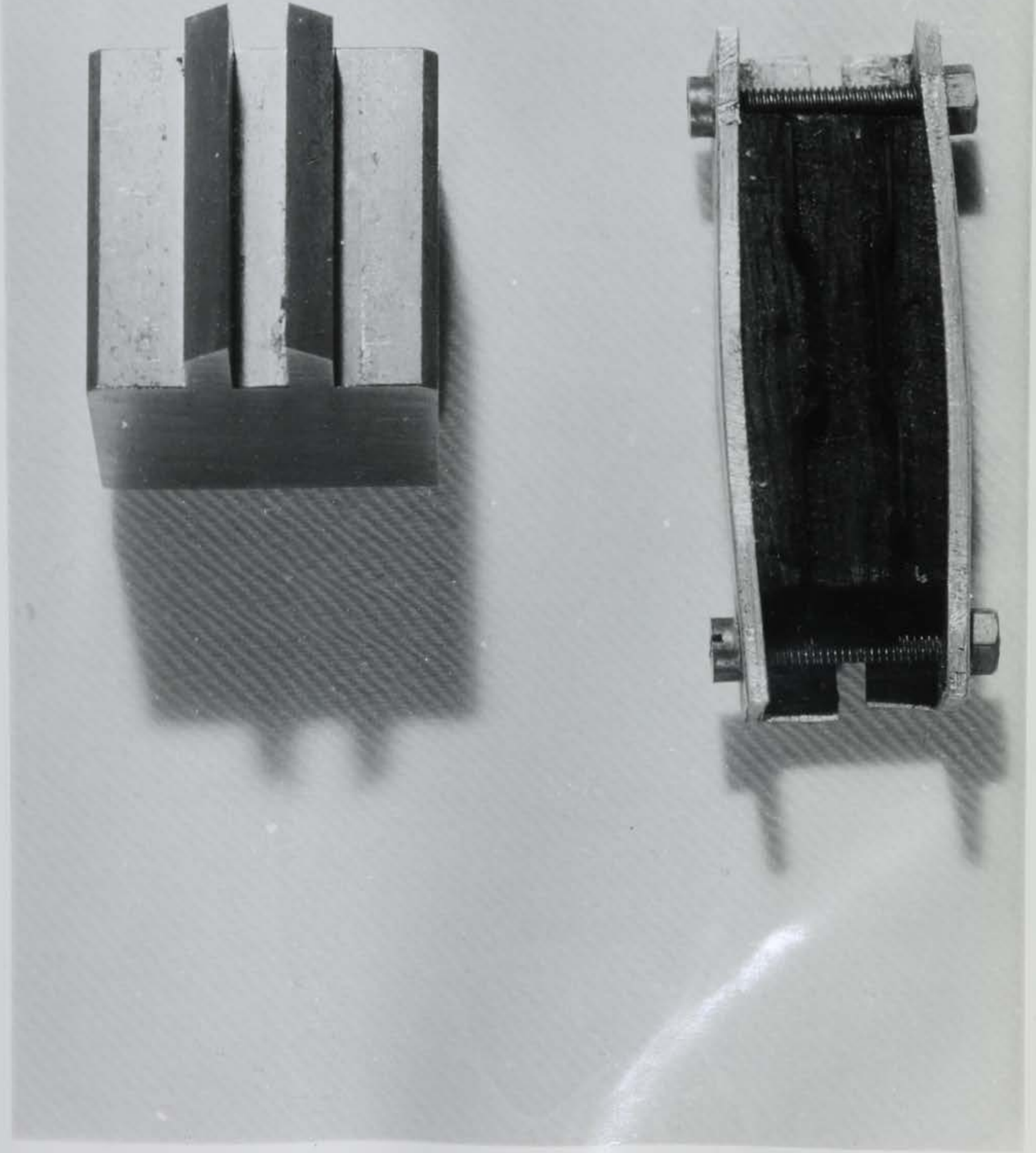
it was found that even at small loads there was a tendency to show signs of premature rupture in the neighbourhood of the jaws - a phenomenon known as 'necking' (Steeds, 1951). This resulted from the jaws crushing the fibres of the specimen and thus causing a weak spot. This gave unreliable results as it was not possible to know at what particular instance during the test necking commenced, and therefore how to compensate for it.

Modified cutter.

The problem of necking at the jaws is common to tensile testing of many materials, e.g. rubber, leather and metals, and has often been overcome by using specimens whose width increases in the region to be gripped by the jaws (Ewing, 1899). In this way even if there was extensive damage to the fibres by the jaws, the tendency to neck was eliminated, due to the reduction in stress.

A suitably shaped cutter (plate 2) was produced for skin, according to the B.S.S. 1910, having two blades forged from sharpened tungsten steel strip, secured parallel to each other in a wooden block backed by a steel percussion plate (see figure 5 for shape and dimensions). This cutter was found to work as well as the original parallel blade and was used for many hundreds of specimens.

PLATE 2. Dies used for cutting skin. On the left the parallel blade cutter, on the right the modified cutter producing a dumbell shape specimen.



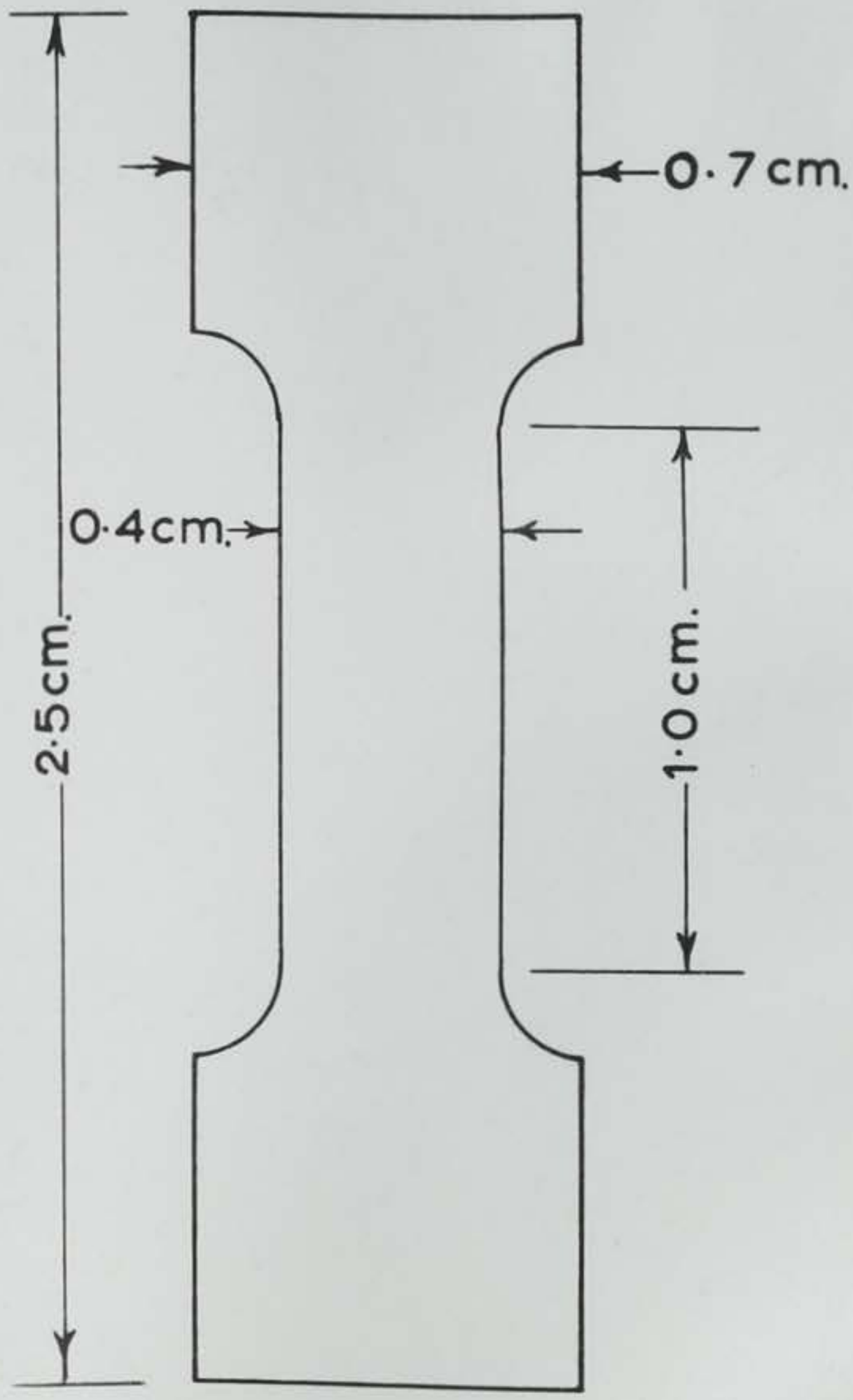


FIG. 5

DETAILS OF SPECIMEN ACCORDING TO B.S.S. 1910

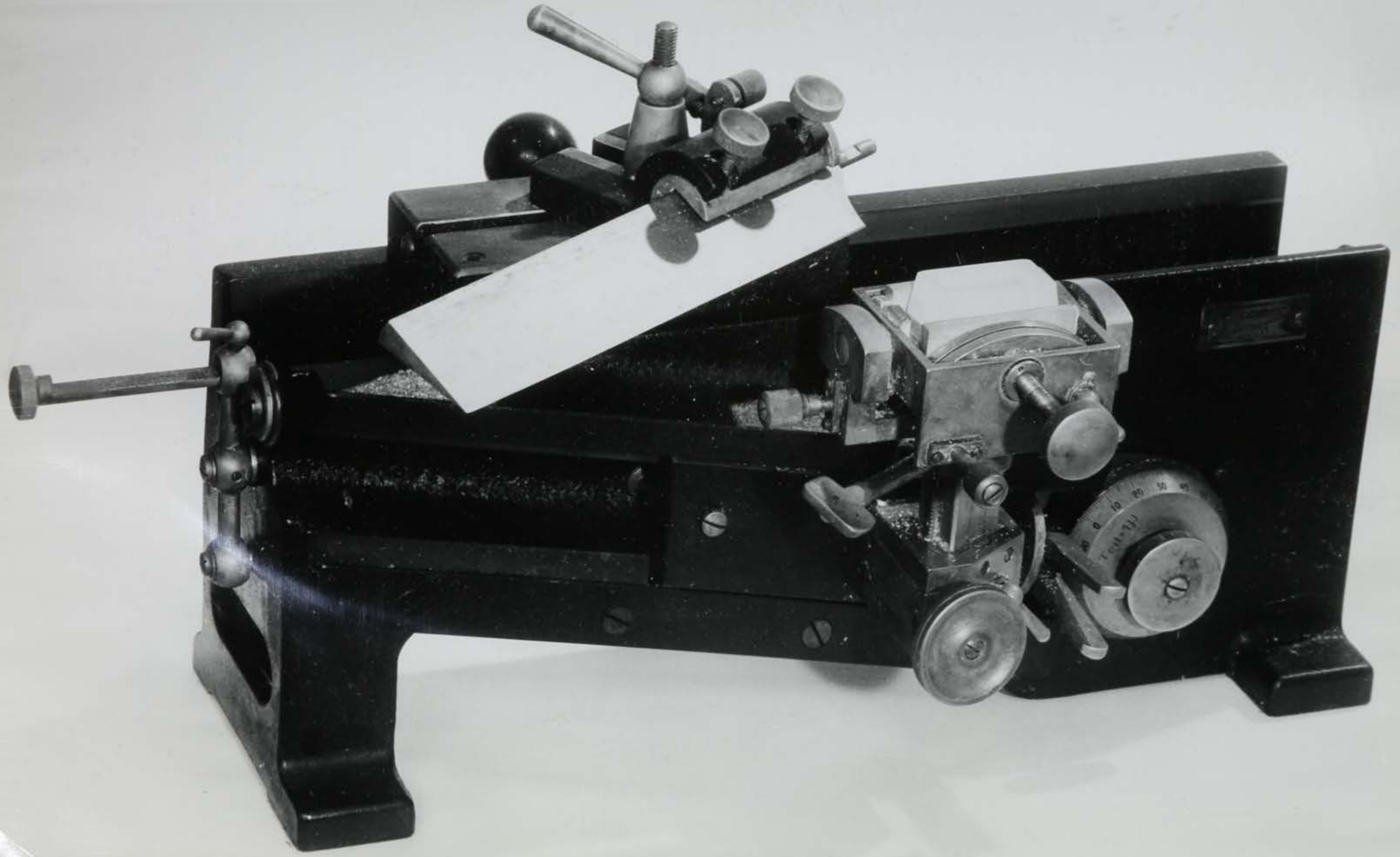
When using the new shape of specimen, it was found that the distance between the jaws was immaterial, and thus the need for length standardisation was eliminated. This was because it could be assumed that the extension took place in the narrow portion of the specimen, which is of constant length. Any change in distance between the jaws was accounted for by the wider portions of the specimens where the extension would be negligible on account of the lower stress.

3. THICKNESS CONTROL

As the thickness of skin varies, it was felt that samples should be cut to a thickness which removed all the sub-cutaneous fat and also part of the dermis. In this way, a comparable specimen consisting of the full thickness of the epidermis, and part of the dermis would be produced.

A microtome of the sledge variety was used and, being heavy and robust, was particularly suitable for this application (plate 3). It had a sharp six inch blade, which moved on slides in a horizontal plane, having a travel of about one foot. The specimen was mounted on a table directly under the blade, and was automatically raised by desired increments between 1μ and 30μ at each stroke of the blade. The table also had provision for

PLATE 3. Microtome of sledge variety.



tilting in the two horizontal planes to achieve alignment of the specimen.

It was not possible to cut the skin in the condition received from the subject due to the mobility of the specimen. In normal microtome work it is common practice to set the specimen in a hard wax, which penetrates it and forms a matrix to hold the soft tissue rigidly while cutting. In the case of skin for rheological tests the use of wax was prohibited since it would not be possible to ensure that it had all been removed from the specimen after cutting and the application of hot wax to skin may cause irreversible changes.

A similar problem to this was encountered by archaeologists in an endeavour to preserve waterlogged wooden objects from prehistoric burial sites in Switzerland (Annotation , 1960) and a satisfactory answer was found using synthetic polyethylene glycol waxes. These waxes - products of the petroleum industry - possess the characteristic physical properties of ordinary waxes, but have the unusual property of being soluble in water. They are available in a polymeric series, ranging in physical consistency from soft materials, rather like Vaseline (molecular weight 600) to hard waxes similar to typical

paraffin waxes (molecular weight 4000). The required properties of the glycol for the present work were a melting point 30° C and the consistency of hard soap at room temperature. The first products tried were manufactured by Shell Chemicals Ltd. and were supplied in molecular weights of 600, 800, 1500, 4000. It was necessary to blend these to produce the desired properties. Many of the mixtures had a granular structure and were unsuitable. A 50:50 mixture of the glycols of molecular weights of 600 and 4000 fulfilled the requirements of melting point and consistency.

The skin was immersed in the warmed glycol mixture, but on cooling the glycol did not solidify in the immediate vicinity of the specimen. It appeared that the water content of the skin was sufficient to dissolve the glycol.

Another compound investigated was soluble nylon, manufactured by I.C.I. under the name of Maranyl C109/P0V55. This product overcame the difficulties of the previous one. It penetrated the skin and solidified it. Unfortunately, the compound was soluble only in 70% alcohol, which had adverse effects on the skin and so this product was discarded.

Frozen specimens.

To achieve rigidity of the specimen the effect of

freezing was studied. A piece of skin, which had previously been moistened with normal saline solution, was placed on a hard-wood block, and cooled to about -10° C in a refrigerator. The freezing process was carried out slowly over a period of 12 hours, to minimise the thermal strains set up in the specimen, which may cause breakdown of the structure. Although the specimen could be easily cut with the microtome it quickly thawed and came unstuck from the wooden block.

Small boxes with an open top were therefore constructed of various materials (figure 6), specimens were placed in them, surrounded by water and frozen. Such boxes were placed directly on the microtome, and sliced down with the specimen. A much higher thermal inertia was automatically achieved, enabling the specimen to remain in the frozen state longer.

Experiments with boxes made from plaster of paris, candle wax and other waxes demonstrated that a wax of melting point 56° C was the most suitable material. With it boxes could be produced which were hard enough to keep their shape, cut easily, did not crack or become over-hard on cooling, and were cheap, disposable and easy to produce accurately in large quantities.

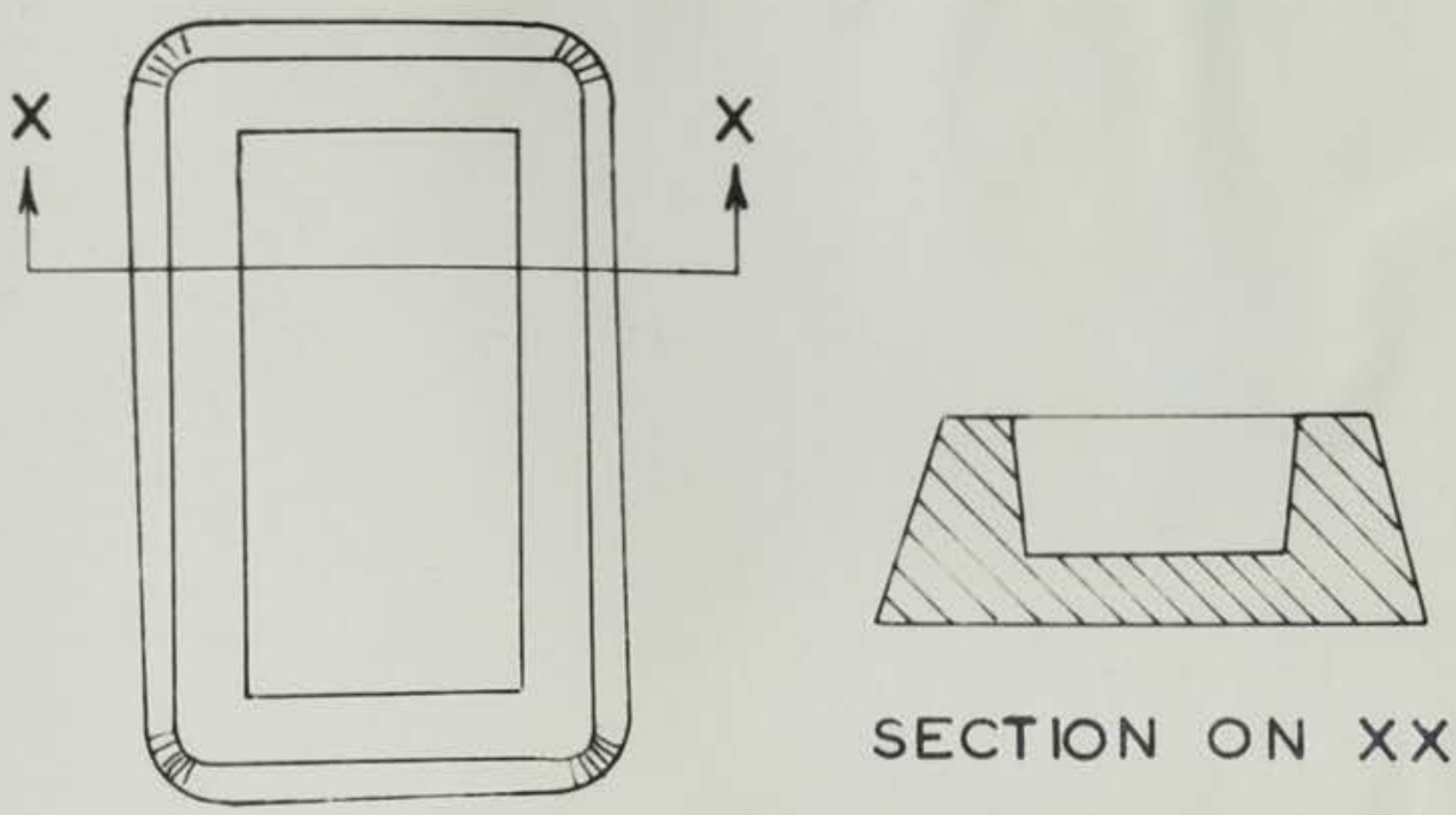


FIG. 6

DETAIL OF WAX BOX 1·25 FULL SIZE

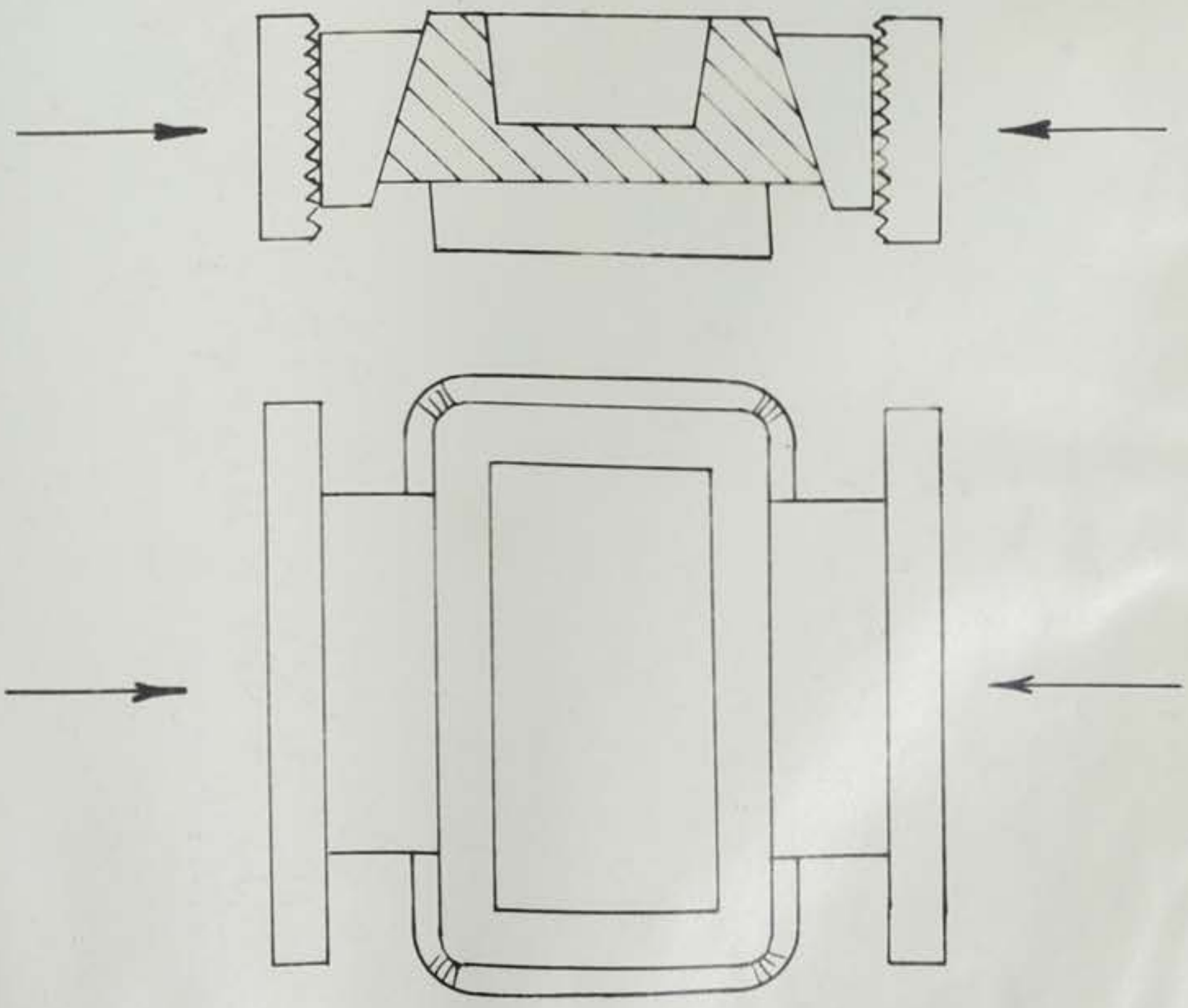


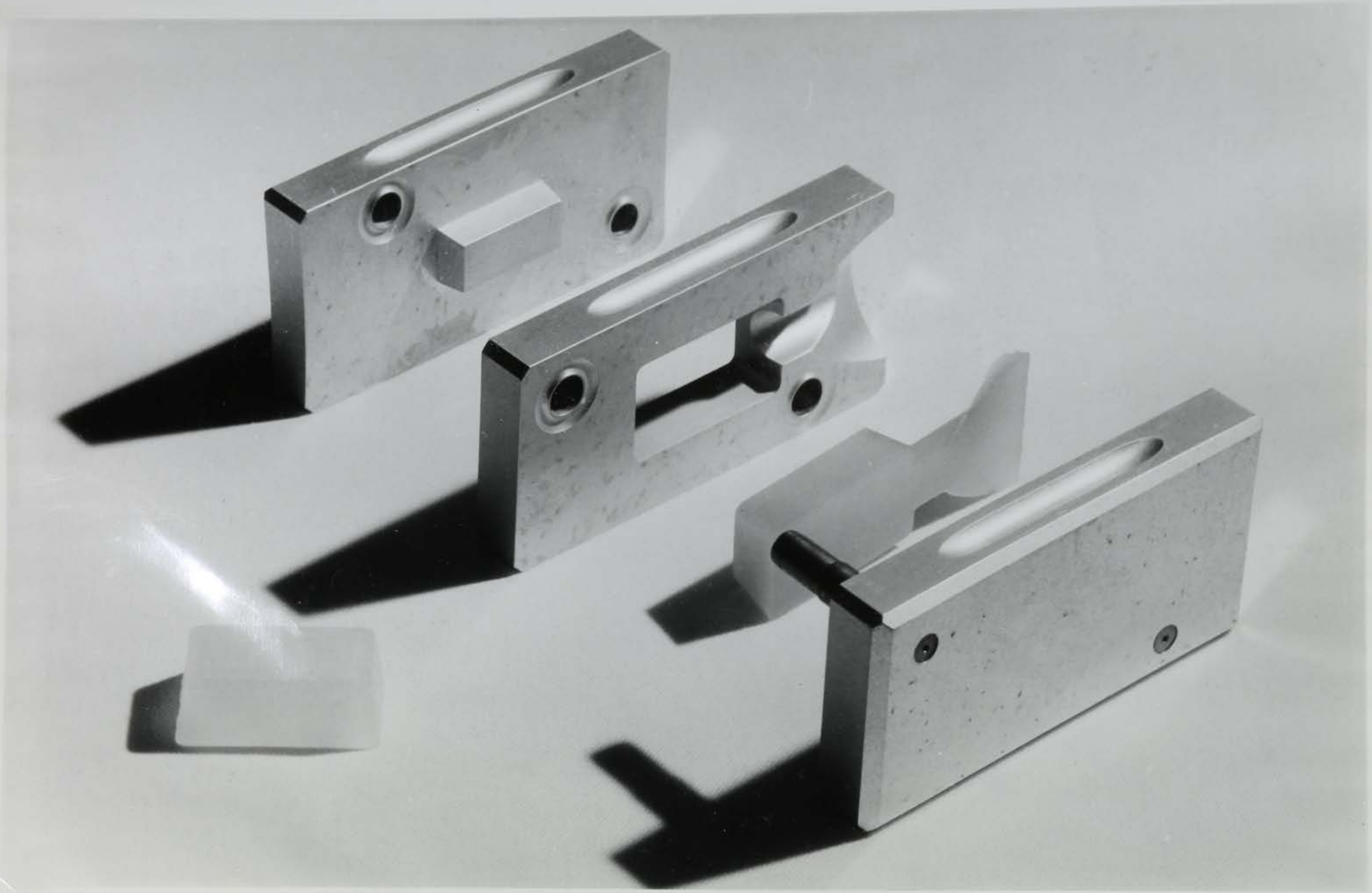
FIG. 7 WAX BOX MOUNTING

The boxes were cast in a mould which could be used many times. They had tapered sides which assisted in extraction from the mould, and which pulled the box down squarely on to the platform of the microtome, when used in conjunction with tapered wedges of similar angle (figure 7).

A mould was made of glazed consolidated asbestos. This mould consisted of three parts, a plane bottom, a top having a block the shape of the inside of the box mounted on it, and a piece having a hole in the form of a truncated rectangular pyramid removed from its centre, to shape the sides of the box. With this mould the desired parallism and reproducibility of $\pm$ 0.001 inch could be obtained. Later a more robust mould was produced to the same design in aluminium (plate 4) which had a much higher thermal capacity, enabling the boxes to be cast more rapidly.

Prior to cutting with the microtome, the boxes were removed from the refrigerator and placed on a sheet of aluminium, which rested on a lump of solid carbon-dioxide. This lowered the temperature of the box to an extent that the skin remained frozen for the whole cutting time. Conditions were further improved by initially cooling the blade and table of the microtome with solid carbon-dioxide.

PLATE 4. Mould for casting wax boxes.
A freshly produced box is shown
and one which is ready for use.



To overcome any tendency for the specimen to float when the saline was added, the skin was first soaked in saline, then placed in the box and a small amount of saline added (0.5ml.). The skin stuck, epidermis downwards, to the bottom of the box by surface tension. After freezing for an hour, the box was filled with saline, and the freezing continued.

It was discovered that if the skin was hairy (e.g. from the forearm of a man) the hair supported the skin and prevented it coming in contact with the bottom of the box. Attempts to shave the hair from specimens removed from the subject were not successful and it was found best to shave the skin before cutting it from the body.

"Slab cutting" technique.

In many experiments using autopsy samples the piece of skin obtained was of sufficient size to enable a considerable number of specimens to be cut from it. This was beneficial for checking reproducibility and for observing the effect of changes in experimental technique, using some specimens as controls.

The preparation of many specimens from a single piece of skin was time consuming in that for each specimen a wax box had to be made, the specimen cut to size, mounted and cut down to thickness on the microtome. This

difficulty was overcome by the following method.

A piece of consolidated asbestos 8 x 3½ x ½ inches, was mounted on the platform of the microtome (figure 8) and a 5/8 inch layer of hard wax was cast on to its upper surface; this adhered firmly. To level the platform accurately, a factor of critical importance when using such a large area, a thin film was cut from the surface of the wax before use, so that it would be exactly parallel to the blade in width and stroke. The platform was then raised to compensate for the amount of wax removed (plates 5 and 6).

A second slab of consolidated asbestos of similar dimensions to the above was prepared, parallel to within 0.001 inches. On its surface a piece of skin was laid with its epidermis downwards. A low wall of "plasticine" surrounding the skin was fixed to the asbestos (figure 9). The enclosed area was filled with normal saline and the slab placed in the refrigerator at -30° C for one hour.

The slab with the skin, now adhering firmly to the surface, was placed on the wax slab on the microtome and clamped by bolts at its corners. Shaving were taken, cutting the plasticine and ice and removing excess fat from the skin, reducing it to a predetermined thickness (figure 10).

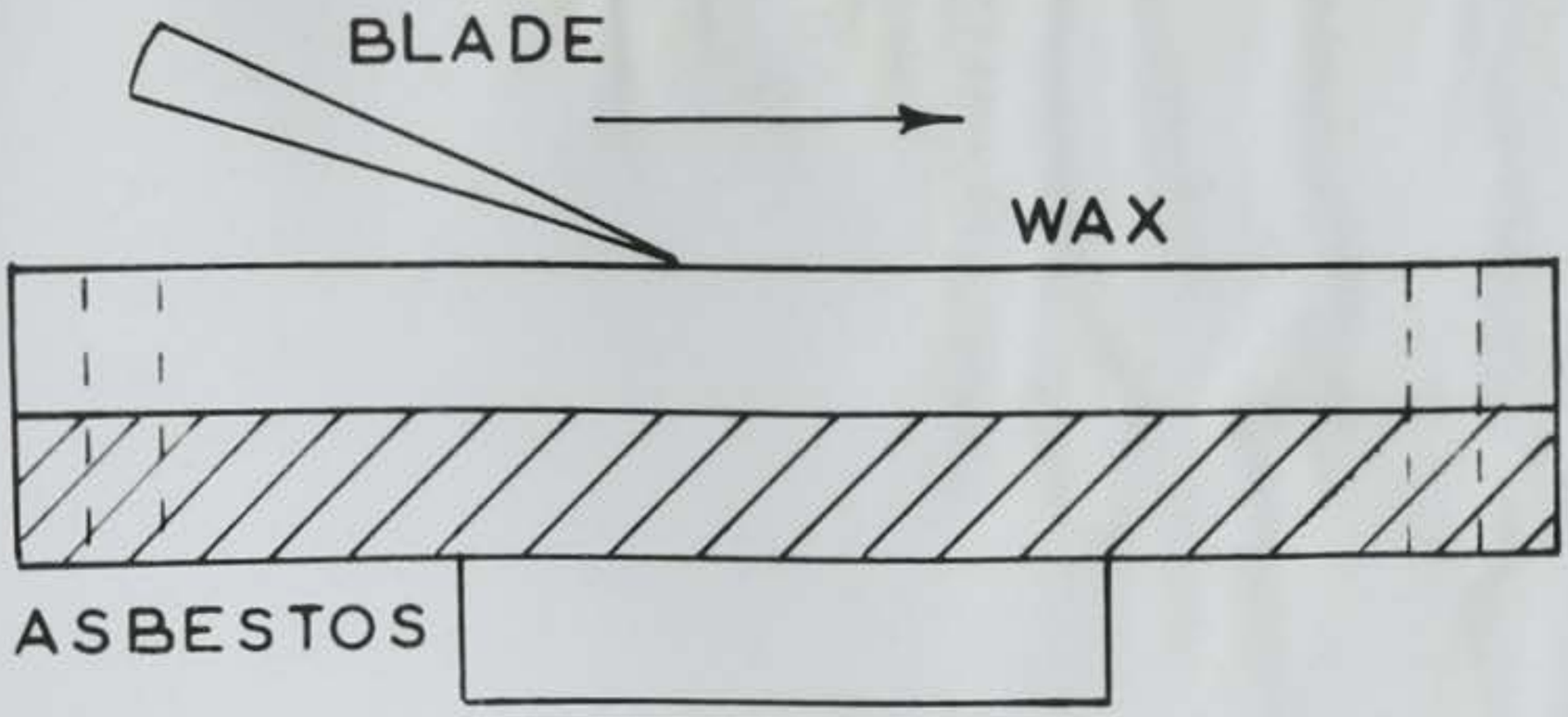


FIG. 8

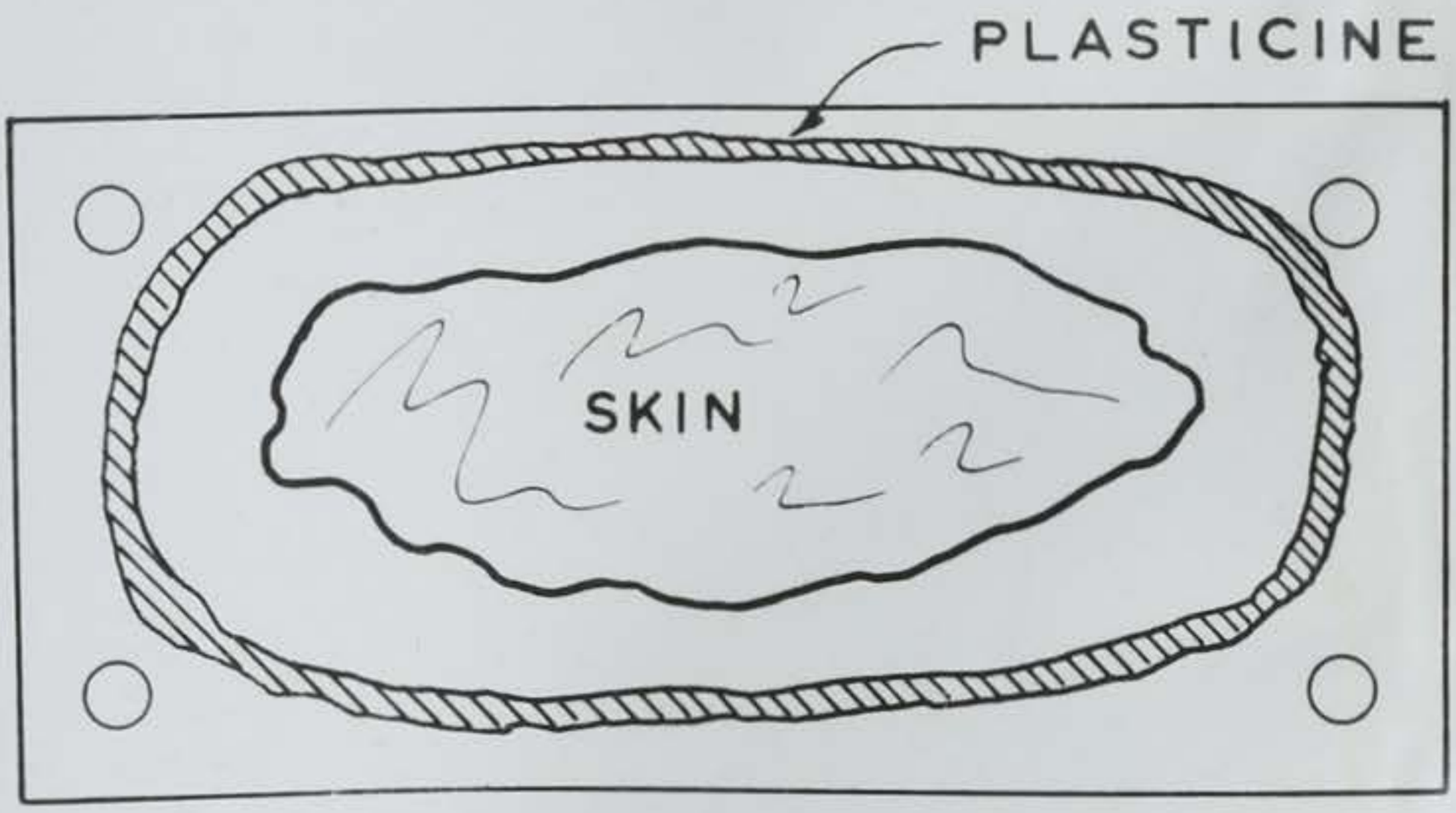


FIG. 9

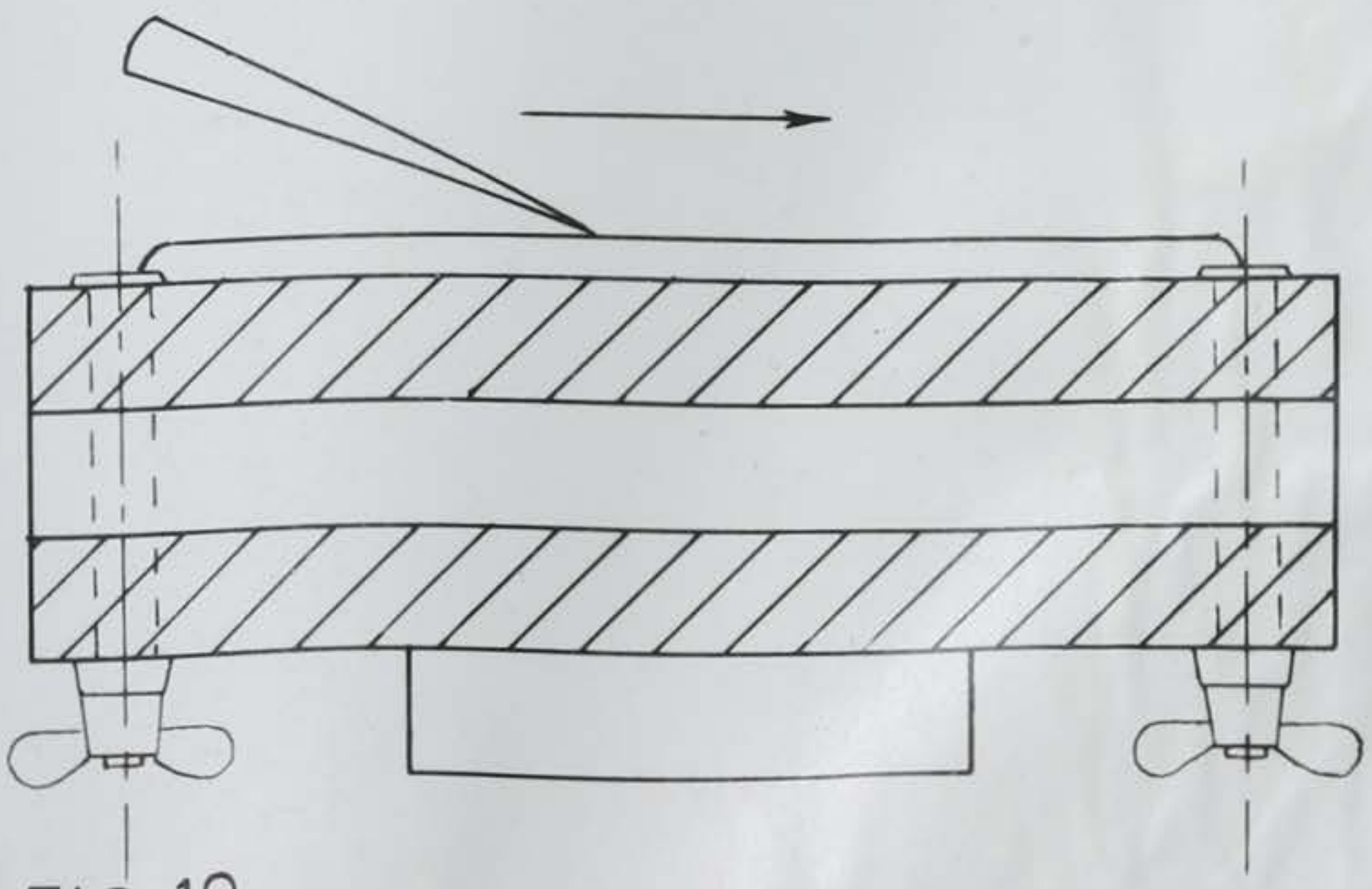


FIG. 10

SLAB CUTTING

PLATE 5. Microtome with cast wax slab on platform and asbestos on which specimen is mounted at the side.

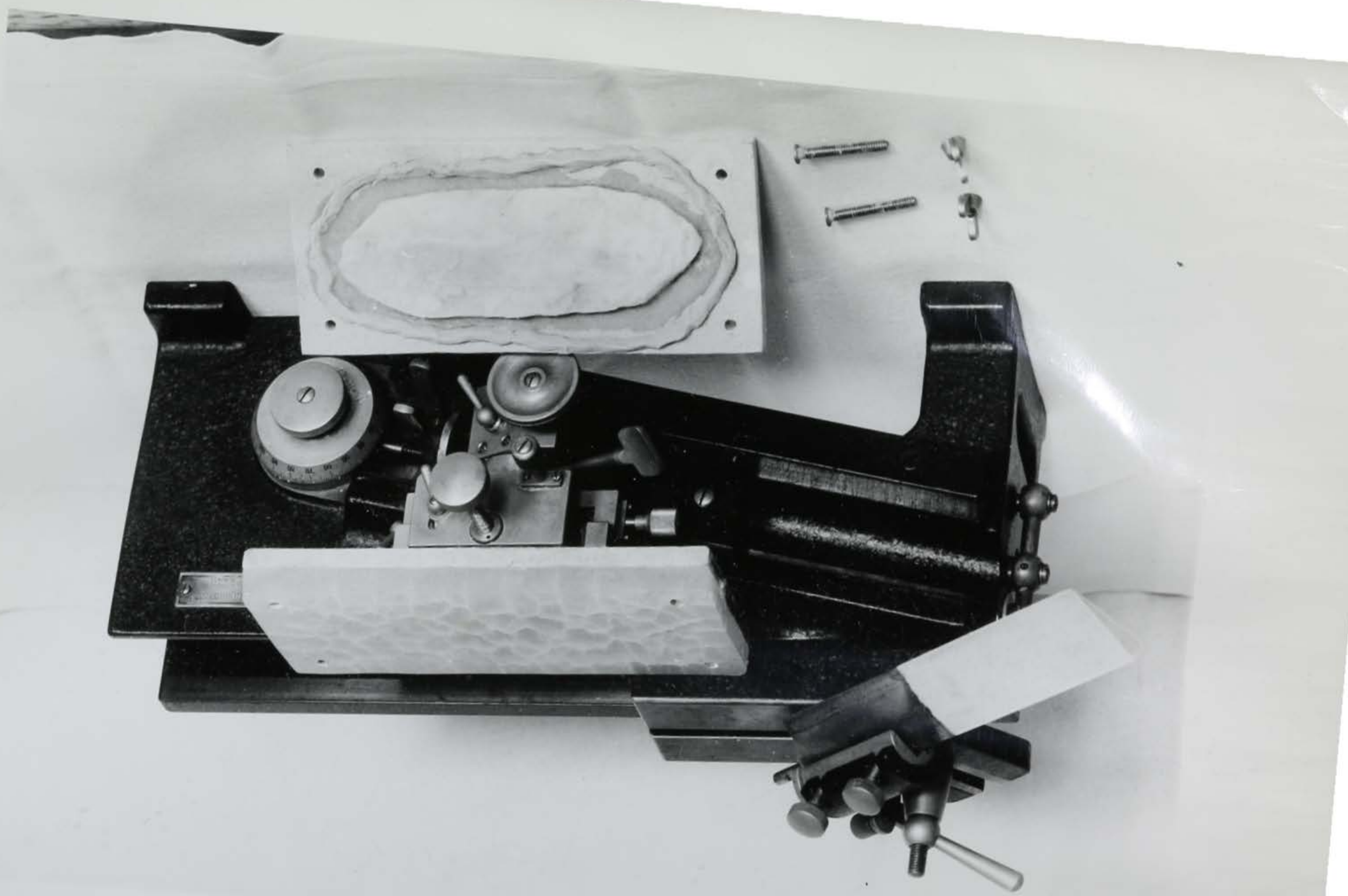
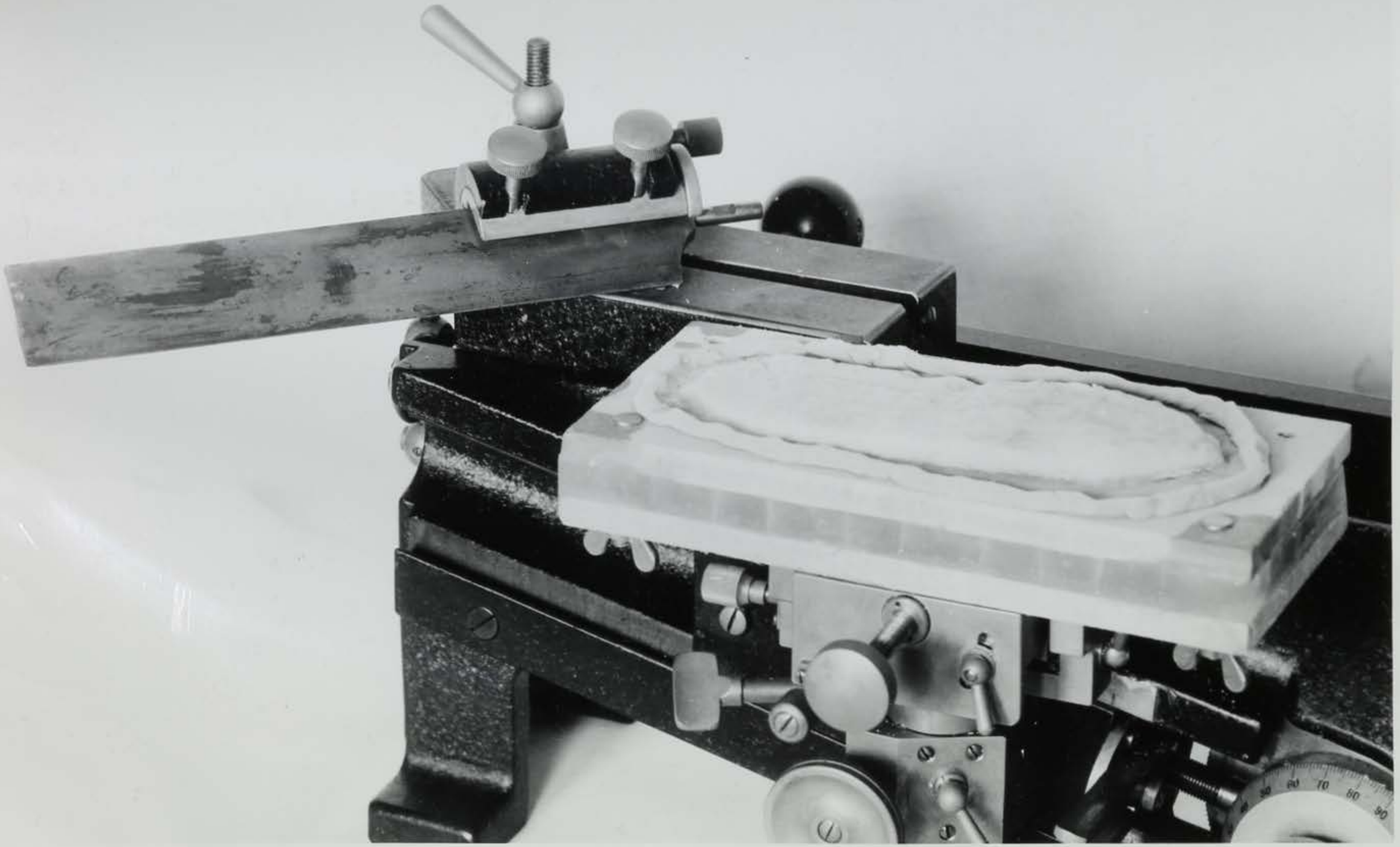


PLATE 6. Microtome with asbestos mounted on platform.



When the skin was cut to the required thickness, but while still frozen, its slab was removed from the microtome and the specimens were cut by pressing the die through the skin into the asbestos. It was found that in addition to being more rapid than using wax boxes there was less skin wasted, since specimens could be cut very close together. Another advantage was that several pieces of skin could be placed on the same slab and cut down together.

The slab cutting method proved advantageous where large numbers of specimens had to be prepared from a single piece of skin. However, for smaller samples of skin, such as biopsy specimens, the wax box method proved superior in that more support was given to the specimen while cutting.

B. SKIN EXTENSION APPARATUS

1. THE INSTRON TESTER

For preliminary extension work on skin an Instron tester (Howard, 1960; Booth, 1961), belonging to the Department of Textile Industries of Leeds University, was used by kind permission of Professor C.S. Whewell and Mr. W.J. Onions.

The Instron tester is a constant rate of traverse machine of low inertia in which a strain gauge in a Wheatstone bridge circuit forms the basis of load registering device. The machine consists of two main parts, one for testing the sample, the other for computation of results.

The instrument incorporates a highly sensitive electronic weighing system with load cells employing bonded wire strain gauges for detecting the tensile or comparison load applied to the sample under test. The pulling jaw was attached to a moving cross-head through a gear box which provided for alterations in speeds. The recorder chart was driven synchronously with the cross-head. A wide variety of speed ratios were obtainable, thus allowing a large choice of sample extension amplification factors. The machine was supplied with interchangeable cells, which gave full scale sensitivities from 100gm. to 100kg. according to the cell. However, the system itself exhibited no mechanical inertia and consequently did

not influence the properties of the sample through its own action. The load cell was fixed on to the upper cross-head and the top jaws were attached to the cell spindle by a flexible coupling.

When a load was applied to the cell, it caused proportional change of resistance of the strain gauges, which were arranged in bridge circuit, and excited a stabilising oscillator. The circuit amplified the resulting signal, and incorporated a flexible means of balancing the gauges, so that varying weights of jaws, fixtures and samples could be compensated for. The amplifier sensitivity was changed in callibrated steps of 1 to 5, 10, 12 and 50. Each cell provided a number of full scale load ranges.

The zero and balance controls had different effects on the load weighing system. The zero knob controlled the recorder pen position and was not affected by changes in load sensitivity through either the load selector or the callibration controls, but the balance controls operated the load cell circuit. This factor was important in the balancing and callibration procedure.

There was a range of chart and cross-head speeds, each system having its appropriate change points plus various gear wheel combinations.

Procedure for use.

The machine was switched on to allow to warm up. The appropriate load cell was selected according to characteristics of the specimen to be tested. The capacity of the cell should be greater than the expected load. If a choice was possible between two cells, because of overlapping ranges, then the higher cell was selected and used in its lower ranges. The coupling and jaw was attached, and all minor switches on the recording and cross-head panels were checked to see that they were connected to the appropriate cell. The required chart and cross-head speeds were then selected and the gearing chosen accordingly.

The procedure was to balance and callibrate the system. The load cell isolator was turned on (red light goes out), the zero button was pressed and the zero control adjusted until the pen was at the desired zero point. When the button was released, it was likely that the pen would go off scale, due to the unbalance of the load cell circuit. Coarse and fine balance controls were turned until the pen was brought back on the scale and it coincided with the previously adjusted zero. The load selector was set first at a higher range and gradually turned to the most sensitive range as the balance point was reached. Final balance adjustment was made and the load selector set at the most

sensitive position. There should be no movement in the balance as the load selector was turned to the positions, provided that both the zero and balance points had been adjusted to be at the same point on the chart. The pen motor was switched off and the red light came on as the appropriate callibration weight (100 grams for cell B) was hung from the top jaw. The red light was turned off to restore sensitivity to the recording system and the load selector turned to its most sensitive position. The pen was adjusted by the callibration control until it was in the desired position on the chart.

The sensitivity of the load weighing system was calli-brated for all available ranges within its range capacity, as selected by the load selector switch. The callibration weight gave full scale deflection at standard load ranges. The desired load range was selected and the gauge length and return dials set as required; the bottom jaws were moved down and stopped at the required initial distance at which the sample was to be tested. The pen motors were then switched off (the red light went on) to reduce the sensitivity of the mechanism while the specimen was mounted between the jaws; the pen and chart motors were then turned on simulta-neously and the desired stress strain curve produced on the recorder.

2. THE SKIN EXTENSOMETER

Preliminary work on the Instron tester determined the general characteristics of skin, but it was found that it was too cumbersome and not flexible enough to carry out the proposed test programme. A machine was therefore designed and constructed specifically for testing skin.

Requirements.

Ease of instrumentation and computation dictated a system using a constant rate of strain, rather than constant rate of stress.

The machine had to be capable of gripping a specimen, of extending it at any one of a range of constant velocities, and of indicating the resulting changes in tension, up to a maximum of 2kg. Automatic recording of results in the form of a stress strain curve would facilitate the acquiring of precise data, particularly at the upper end of the velocity range.

A number of load ranges had to be provided to enable the optimum curve configuration for analytical purposes to be obtained, and damping had to be adequate to permit switching from one range to another, without loss of response, while the test was proceeding.

Each element of the electronic system had to be stable in zero and gain, with respect to time and changes of temperature, this being achieved, if necessary, by automatic

stabilisation and compensation circuits.

An overall maximum permissible error of ± 1% could be tolerated.

Principle.

In view of the small loads involved in extending a sample of skin an electro-mechanical (rather than mechanical) system was devised.

The specimen was mounted between two sets of jaws, one vertically above the other. The lower jaw was mechanically coupled to the chart drive system of a strip chart recorder. Downward motion of this jaw extended the specimen by an amount proportionate to the motion of the chart. The upper jaw was suspended from the centre of a diaphragm, the deflection of which was directly proportional to the tension in the specimen. This deflection was amplified electronically to produce a proportionate deflection of the recorder pen.

As the extension of the specimen proceeded, the resultant increase in tension could be plotted on the chart of the recorder in the form of a stress strain curve.

Extensometer.

The mechanism of the extensometer can best be appreciated by examining figure 11 and plates 7, 8 and 9.

A sturdy aluminium tube A, in the vertical position,

accurately bored, and having a longditudinal slot B cut in it for part of the length, formed the body of the machine. Through the centre of the tube passed a lead screw C, on which ran a brass nut-block K, which was a sliding fit in the bore of the tube. The nut-block was prevented from rotating by the arm D which projected through the slot B and formed a mounting for the lower jaw E; thus as the lead screw rotated the lower jaw was moved ih a vertical plane and extended the specimen H.

The upper jaw F was suspended via stirrup J from the centre of the diaphragm G, which fitted in a circular recess in a projection from the body of the instrument.

Drive system.

Details of the drive system are shown in figure 12. The drive for the extensometer was taken from the chart system, which in turn was powered by a small induction motor.

The lead-screw of the extensometer was coupled to the secondary shaft B, which ran perpendicular to it, by a pair of brass spiral gears A of ratio 1:1. This secondary shaft was situated under the platform, running in two brass bearings, and driven by the primary shaft D via a system of change wheels E (also under the platform) at any one of a number of integral ratios between 16:1 and 1:16. As the

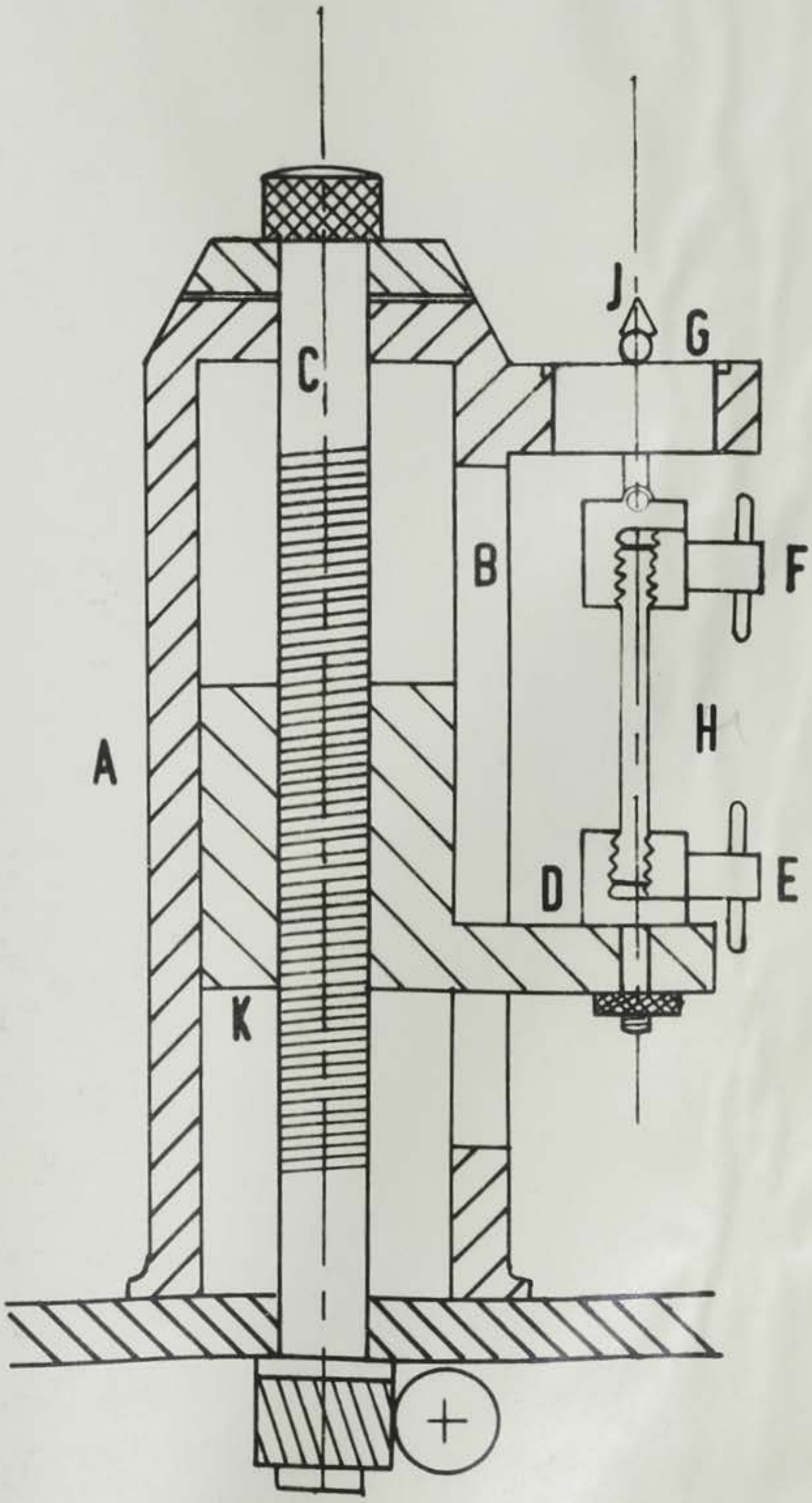


Fig. 11 EXTENSOMETER

PLATE 7. Extensometer with jaws and stirrup removed, and shown separately at the side.

PLATE 8. Extensometer front view.

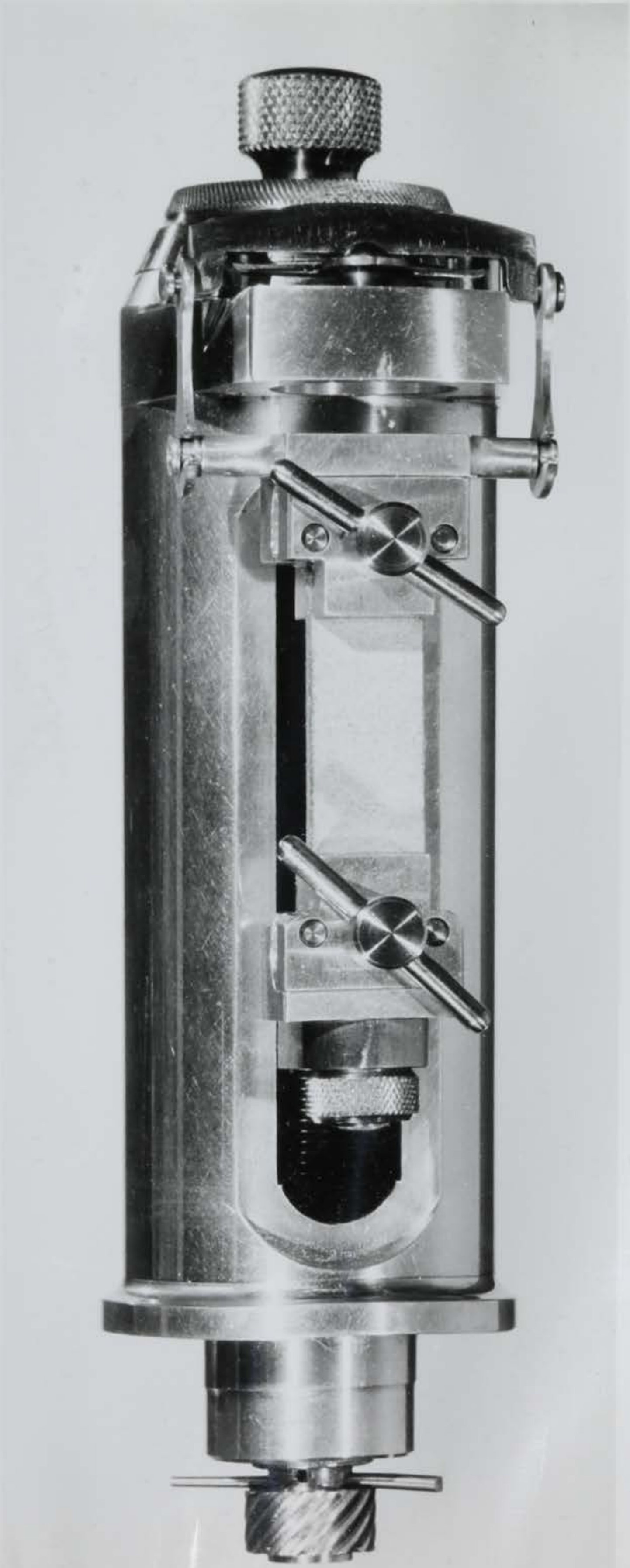
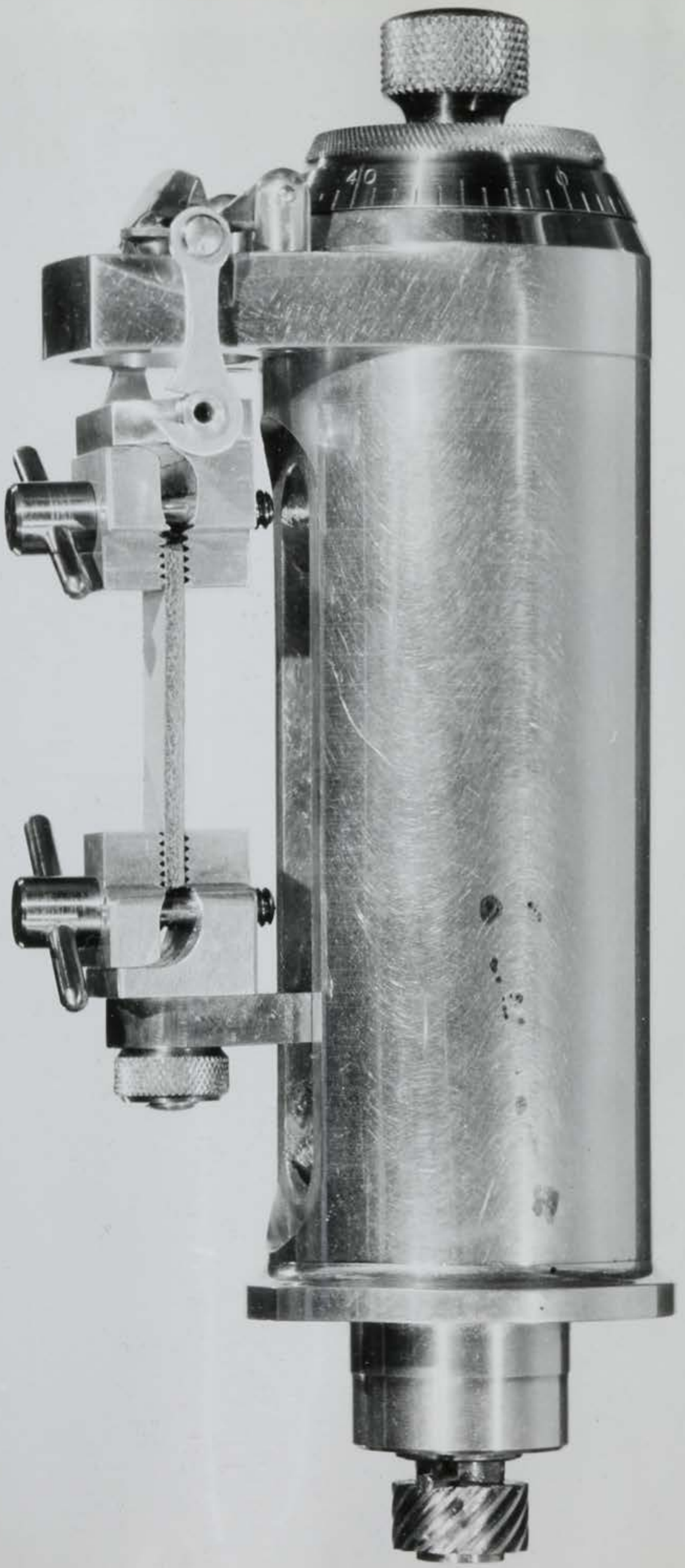


PLATE 9. Extensometer side view.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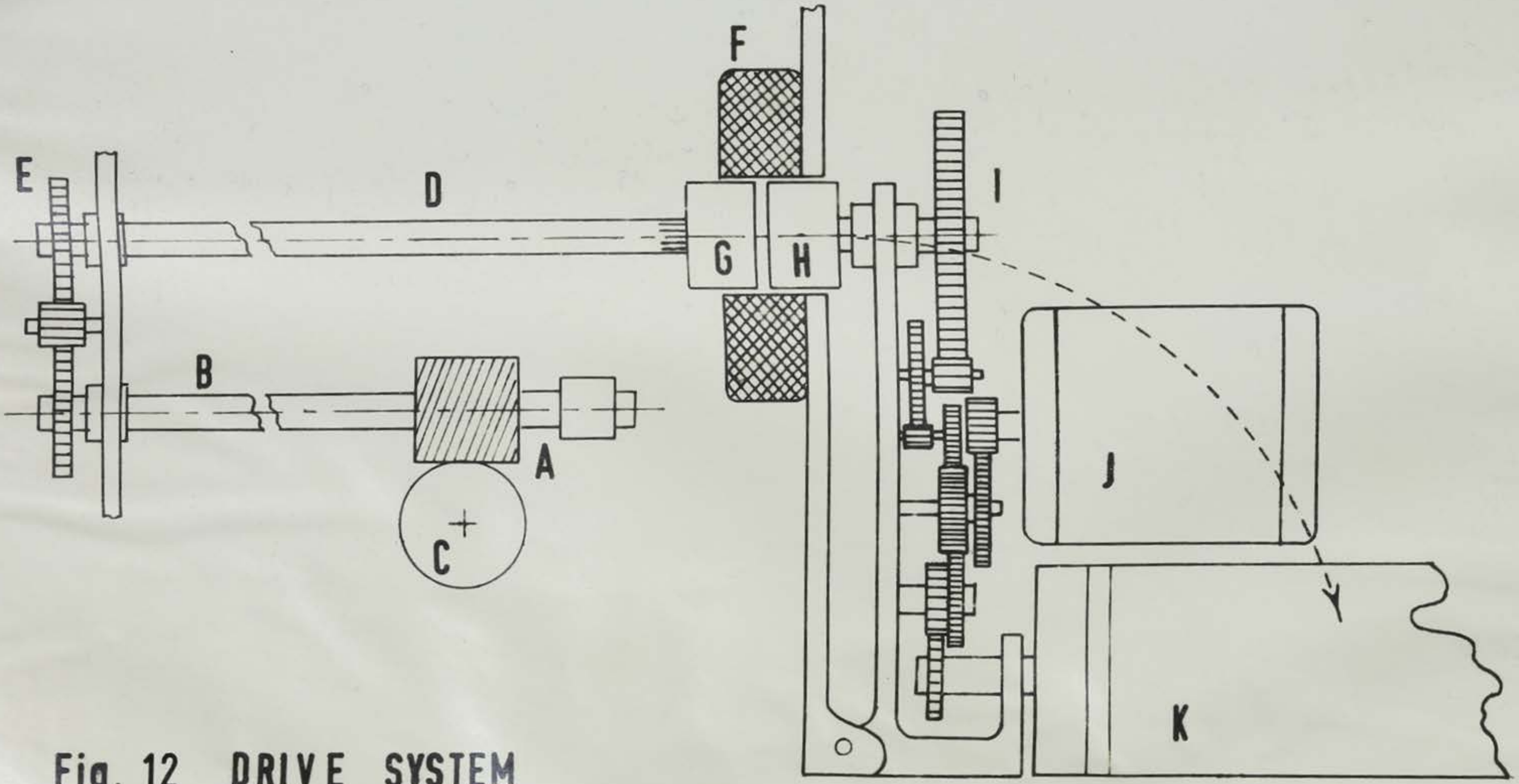


Fig. 12 DRIVE SYSTEM

primary shaft revolved at 4 revolutions per minute these ratios corresponded to rates of extension between 0.0125 and 3.20 inches per minute.

It was necessary that the drive for the primary shaft should be taken through the side of the recorder case to connect with the chart system. Unfortunately, a permanent coupling could not be made, as the whole of the chart mechanism was mounted on a chasis, which was hinged to enable it to swing out from the recorder housing for adjustment and maintenance purposes. This problem was overcome by the use of an electromagnetic clutch, manufactured by Crofts Engineers Ltd. capable of transmitting a torque of 457 oz. ins. which was calculated as being adequate for producing a tension of up to 2kg. at all but the highest of the velocity ranges.

The clutch consisted of an annular coil F, which was mounted on the side of the recorder, housing two steel friction plates in its bore which were pulled together magnetically when the coil was energised. One plate G was fitted with splines on to the end of the primary shaft; this permitted slight axial movement enabling the plates to engage. The other plate H was pinned to a stub shaft I mounted on the chart chasis. The edge of this plate had to be chamfered to enable it to be withdrawn from the coil in an arc, when the chart chasis was swung open (plate 10).

Situated on the hinge side of the chart chasis was the chart drive system. This consisted of a small induction motor J which ran at constant speed, driving the chart roller K via a train of gears, some of which could be altered to give a range of chart speeds between 4 and 16 inches per minute. When used in conjunction with the wheels E this corresponded to a range of extension amplifications from 1:1.25 to 1:1280. A second train of gears of fixed ratio was also powered by the motor J and drove the stub shaft at the required 4 revolutions per minute.

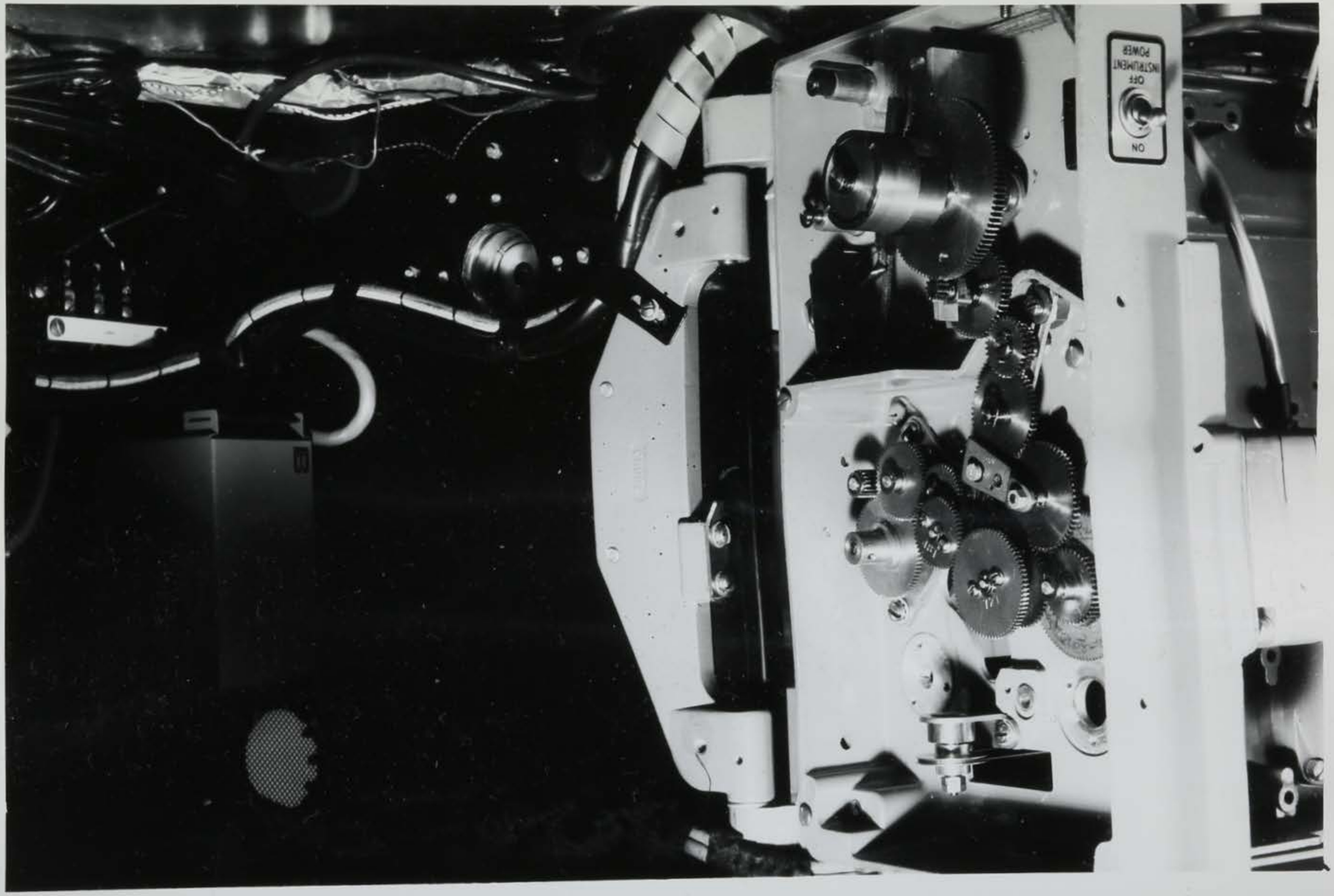
With this system it was possible manually to adjust the initial length of the specimen in the extensometer by means of the knurled knob summounting the thimble and then on energising the clutch a drive was established between the extensometer and the chart drive system.

Lead screw and micrometer head.

The accuracy of the thread on the lead screw and nut-block was a major factor limiting the performance of the machine. It was necessary that these components should be as nearly as possible free from eccentricity and backlash. They were therefore precision made.

A $\frac{3}{8}$ inch British Standard Fine thread was cut to form the lead screw; this had a pitch of 0.05 inch. At the

PLATE 10. Gear train and magnetic clutch mounted on chasis of recorder.


INSTRUMENT
OFF
ON
POWER

upper end of the screw was fitted a thimble divided circumferentially into fifty equal graduations; thus as two revolutions of the screw corresponded to a movement of the jaw of 0.1 inch, each division on the thimble represented an increment of 0.001 inch. The thimble was spring loaded on to the lead screw and could be turned relative to the screw for zeroing purposes.

Jaws.

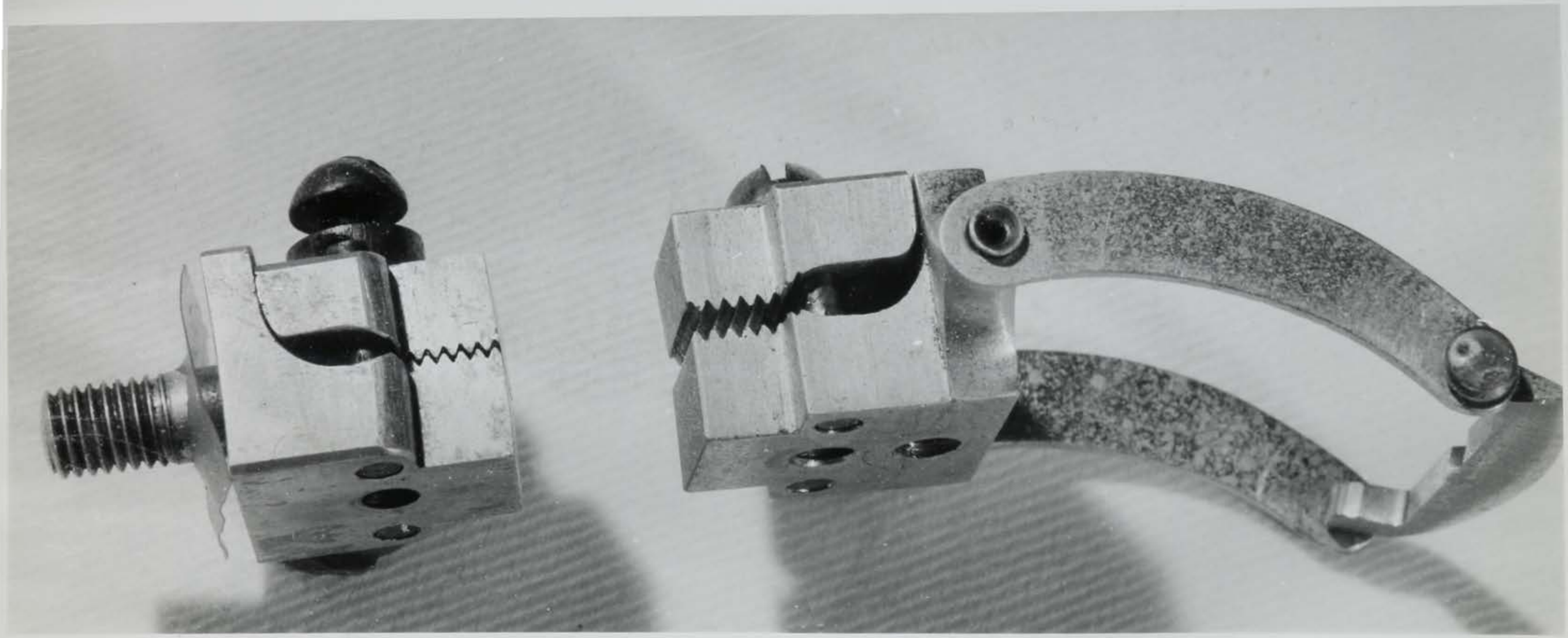
The jaws were required to grip the specimen positively without damaging it, to be as light as possible and to be resistant to corrosion.

It was found that the most suitable type of jaws had two mating serrated faces. These serrations with a V profile had an included angle of 60^{o} and ran across the face of the jaw with a pitch of 3/64 inches, and were slightly radiused at the peaks to prevent cutting (plate 11).

The jaws were made of duralumin and were pulled together with a stainless steel screw fitted with a tommy-bar for tightening, the total weight of each set of jaws being approximately 20gm.

Both sets of jaws were of similar design and differed only in their means of attachment, the top jaw having two trunnions to mount on the stirrup and the lower a screwed stud to secure it to the nut-block arm.

PLATE 11. Jaws of extensometer (note the mating serrations radiused at the peaks).



Diaphragm.

The diaphragm, being the centre of the load measuring system, was bound by critical requirements:- the deflection must bear a linear relationship to the load applied and on removal of the load must return to the exact original position to achieve a reproducible zero. A measureable deflection must be produced for loads as small as 250mg. while no overstraining must occur at maximum load.

It has been shown (Timoshenko, 1954) that the deflection of a rigidly mounted diaphragm is directly proportional to the square of the thickness and that linearity is attainable for deflections not exceeding 0.2 of the thickness.

Diaphragms of various metals were tested including steel, copper-berylium, duralumin and aluminium. The last proved to be the most suitable.

The diaphragm used for the first series of experiments was of the design shown in figure 13. It had an effective diameter of 0.75 inches and a thickness of 0.0085 inches. It had strain gauges bonded to it and was linear up to a load of 250gm.

In later experiments the maximum required load was increased to 2kg. To carry this a diaphragm of the same desigh as the previous one was used, but the thickness was increased to 0.0145 inches, the deflection being measured

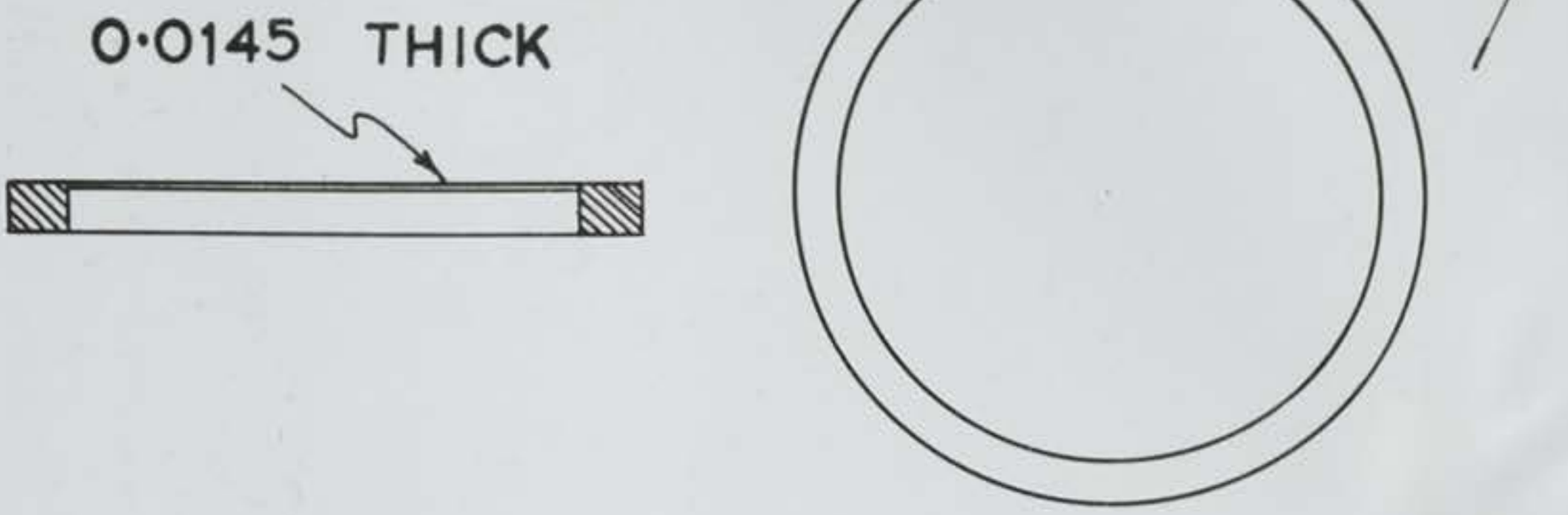


FIG.13

DETAILS OF DIAPHRAGM

FIG.14

CIRCULAR STRAIN GAUGE

inductively.

Load measuring system.

In the first series of experiments the maximum required load was 250gm, and the deflection of the diaphragm was measured by the change of resistance of strain gauges bonded to it. Circular, foil strain gauges, described by Saunders Roe (1962), were used. They had a resistance of 30 ohms per arm (figure 14). The advantage of such gauges was that a high current could be used. However, this caused heating of the gauges, necessitating the provision of a generous heat sink.

The circuitry of the apparatus built is shown in figure 15. The strain gauges were wired in the form of a Wheatstone bridge, balanced by means of a 10 ohm variable resistance and fed directly into the amplifier.

A four transistor amplifier with a gain of 200 (Mullard, 1961) was used, arranged in a "long-tailed-pair" system to give improved stability. A transistor system was chosen, because it could be run from a 12 volt accumulator - a very stable source eliminating the need for mains smoothing and constant voltage control.

OC200 silicon transistors were used, in preference to more common OC72 germanium type, because of the improved temperature characteristics.

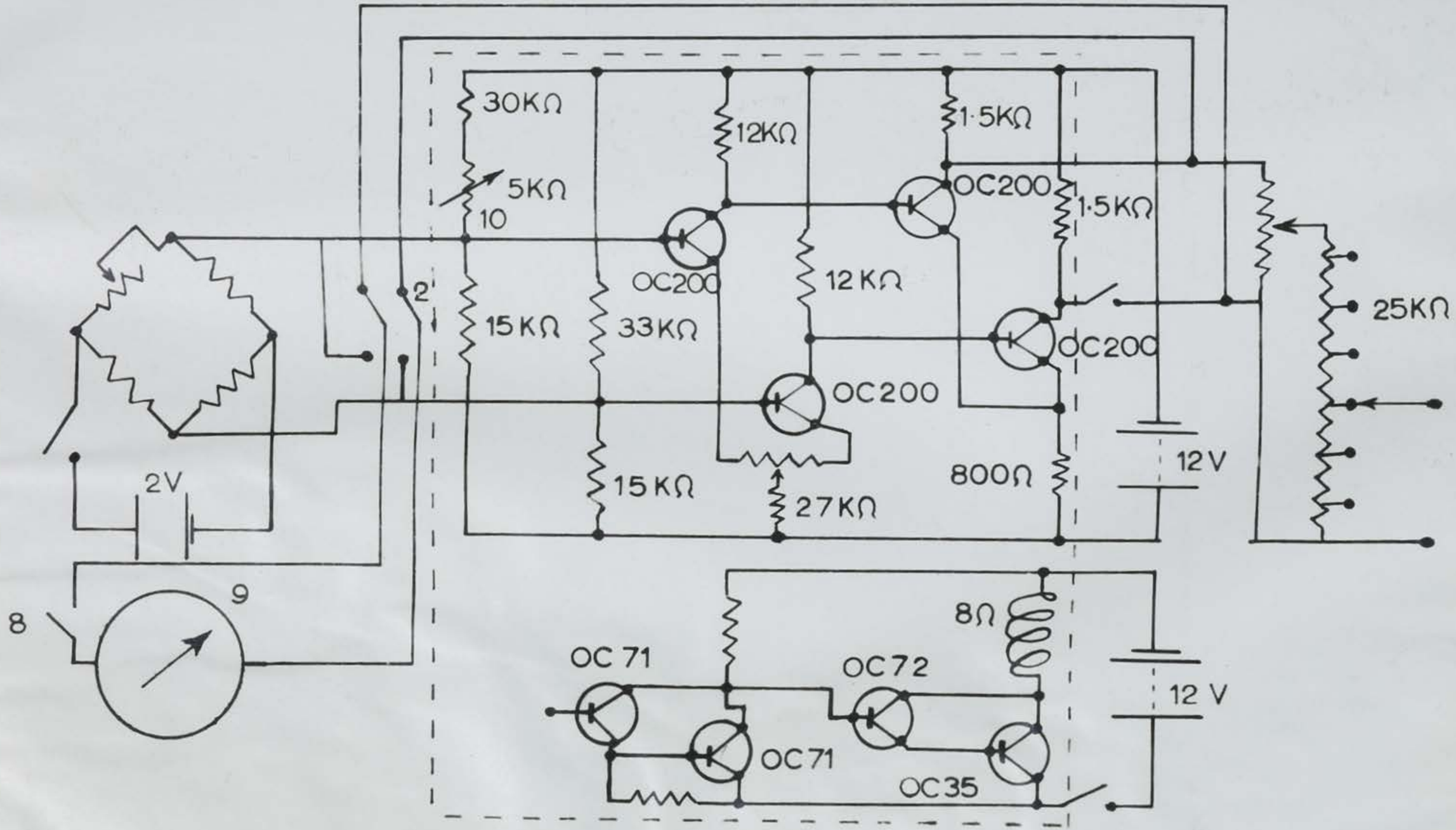


FIG. 15 D. C. AMPLIFIER CIRCUIT

The stability conditions required were so stringent, that in spite of all the precautions already mentioned, it was necessary to embed the transistors in a large aluminium block of high thermal inertia. The temperature of the block was maintained constant within $\pm$ 0.5° C. by a control system shown in the lower half of figure 15. This used the highly temperature sensitive OC72 transistor, embedded in the block, to drive a small amplifier, terminating in an OC35 power transistor. The output of this system was adequate to operate an 8 ohm heating coil wound round the block.

The output of the main amplifier was fed into a cascade potentiometer system, the first stage of which was a 50 K. ohm variable resistance, for calibrating purposes. The second stage consisted of a string of nine pre-set resistors having a total resistance of 25 K. ohm. Each pre-set resistance was adjusted to give a range reducing the maximum output by factors of 1, 2, 4, 5, 10, 20, 40, 50, 100, prior to feeding to the recorder via a multi-position switch.

The complete amplifier and temperature control systems were enclosed in a heat insulating box, which was covered in earthed metal foil for screening purposes. It was also found necessary to screen all leads and batteries to eliminate

the pick-up of electrical "noise".

The variable resistances, switches and also a meter for balancing were mounted on a panel under the machine platform.

Operation of system.

The four systems: bridge, amplifier, recorder and temperature control were switched on to warm up for at least an hour before they were required.

1. The bridge was balanced by putting switch 2 (figure 15) in "BRIDGE" position closing switch 8 and opening 3. Variable resistance 1 was adjusted for a zero reading on meter 9.

2. The amplifier was zeroed by opening switch 7, closing 3 and setting switch 2 at "AMP" position. A zero reading was again achieved by adjusting variable resistances 10 and 4. 10 gave coarse and 4 fine control.

3. To calibrate the instrument switch 8 was opened and 7 closed. A known weight was hung from the top jaw and with variable resistance 6 in position 1 resistance 5 was adjusted to give desired deflection on the recorder.

The apparatus was now ready for use, any slight zero errors on the recorder occuring subsequently were checked with the fine control resistance 10.

Modified load measuring system.

When, due to modifications of the testing procedure, it became necessary to increase the maximum load from 250gm. to 2kgm. it was found that the previously described system was inadequate. This was because the thinnest diaphragm capable of supporting the load was 0.0145 inch thick, and was so stiff that the deflection for a load of lgm. was calculated to be only of the order of 0.00001 inch. Attempts to increase the voltage to the bridge and improve the amplification revealed that the range was so wide as to make strain gauges methods impracticable.

An alternative method of measuring small deflections was therefore developed using an inductive pick-up and measuring bridge (plates 12a, b and 13).

Pick-up.

The inductive displacement pick-up (figure 16) consisted of a cylindrical steel casing A with a small protruding probe K, running axially through the casing. The probe, which carried a small core H of "Ferroxcube" in the middle was supported by thin leaf springs J, ensuring constant transverse rigidity over the whole measuring range.

The core moved in a non-magnetic tube affixed to the casing, on which the primary and secondary coils C and G were wound.

PLATE 12a. Extensometer (side view) with inductive pick-up and perspex tank.

PLATE 12b. Front view of extensometer (modified), enclosed in perspex cabinet.

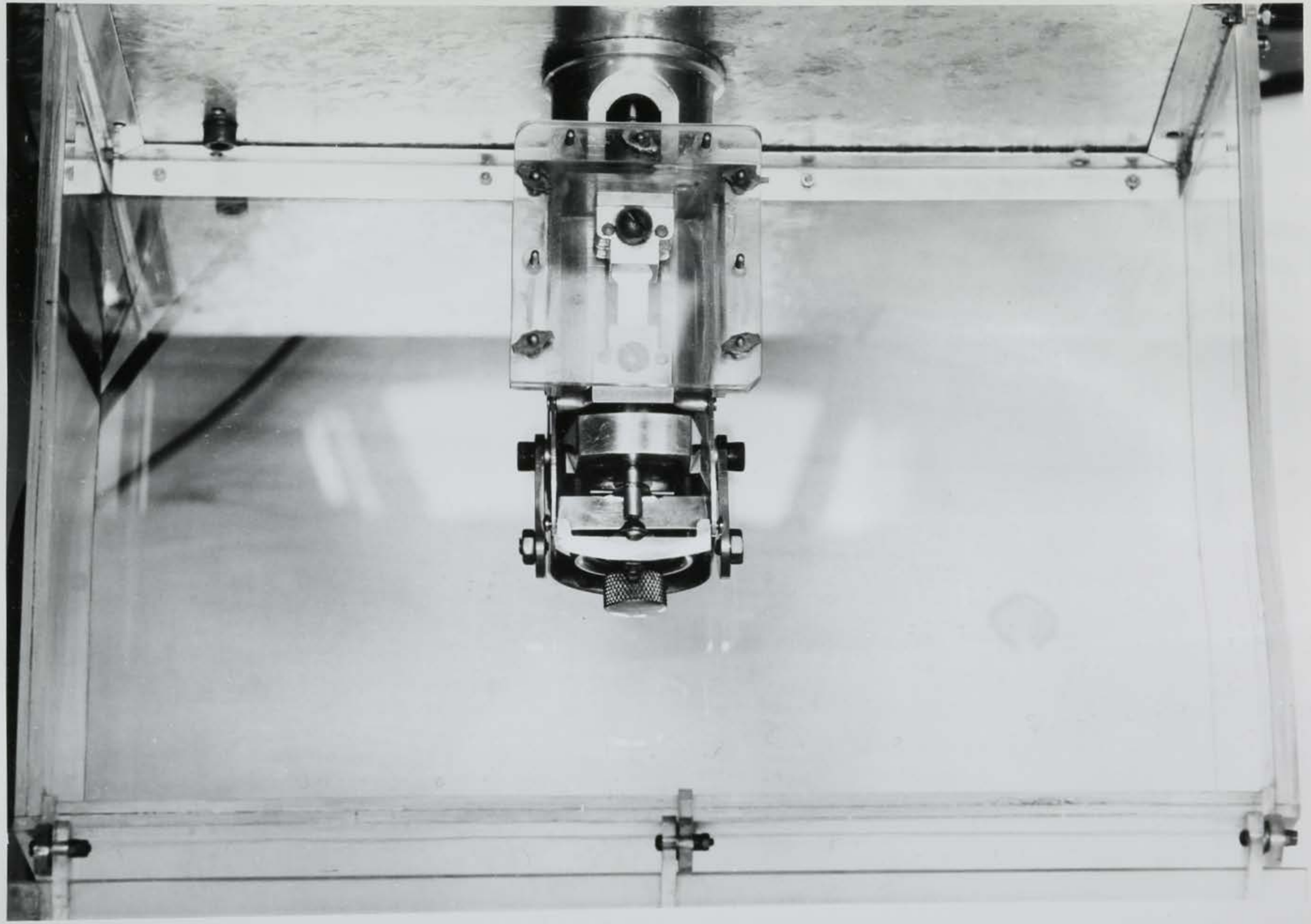
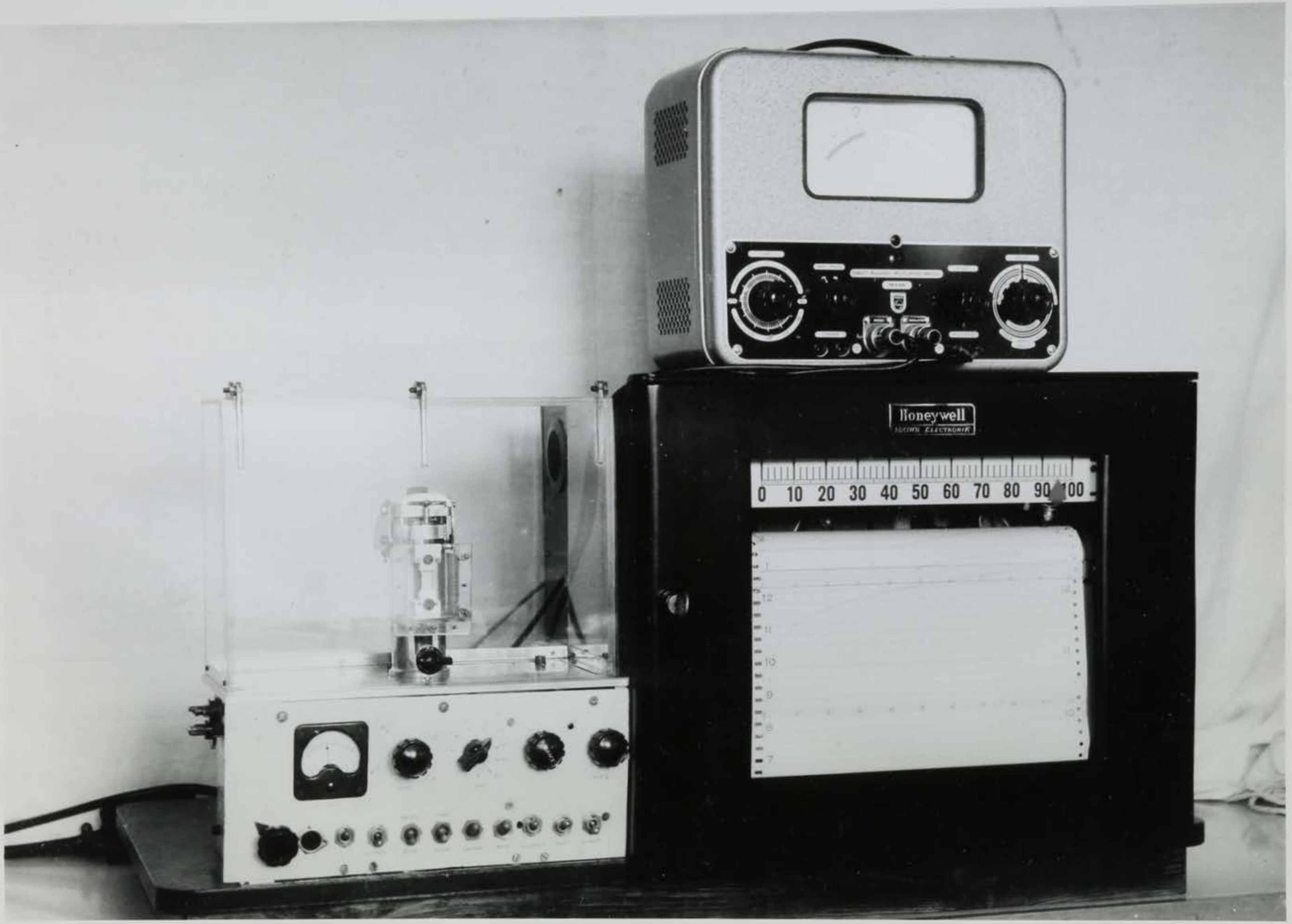
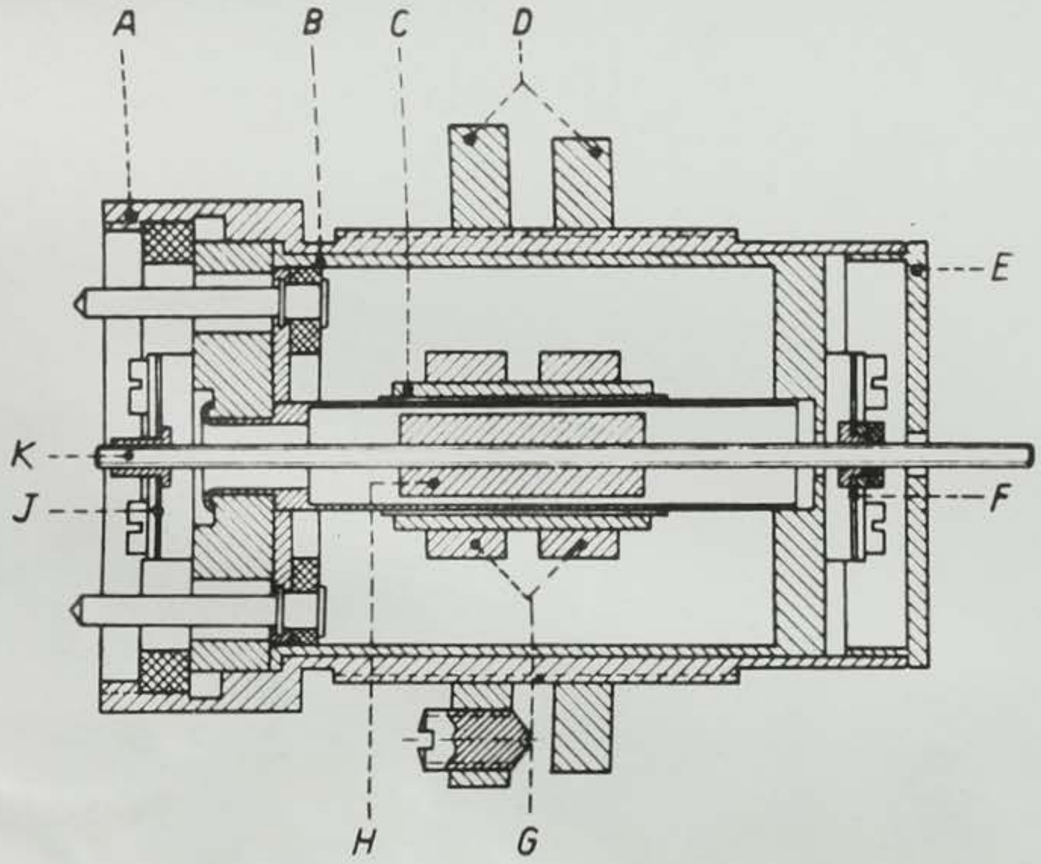


PLATE 13. General view of extensometer and recording apparatus.


Honeywell
0 10 20 30 40 50 60 70 80 90 100



A = steel casing
B = copper can
C = primary coil S_1
D = nuts for fixing
E = cap
F = bearing spring
G = secondary coils S_2 and S_3
H = "Ferroxcube" core
J = bearing spring
K = measuring probe

FIG. 16

INDUCTIVE PICKUP

The principle of operation was to vary the degree of coupling between the primary coil and the two secondary coils, the connections of the latter being so arranged to produce voltages which were opposite in phase on feeding an alternative voltage to the primary. The differential voltage so produced was measured by means of a suitable bridge.

Bridge.

The measuring bridge (block diagram figure 17) consisted essentially of an oscillator, bridge and amplifier.

The primary of the pick-up A was fed with a 4 kilo-cycles per second supply from the oscillator G and the resulting output from the secondaries led to the differential capacitators B which were used for zeroing purposes. The signal then went through a pre-amplifying stage C, fitted with the range switch, and on to the output amplifier D. After the amplifying stages followed a bandpass filter F and a ring-demodulator E. The resulting signal was then fed to a microameter, used for monitoring, which was in parallel with the output terminals.

Prior to supplying the recorder the output from the measuring bridge was fed to the original cascade potentio-meters used for calibrating and range switching.

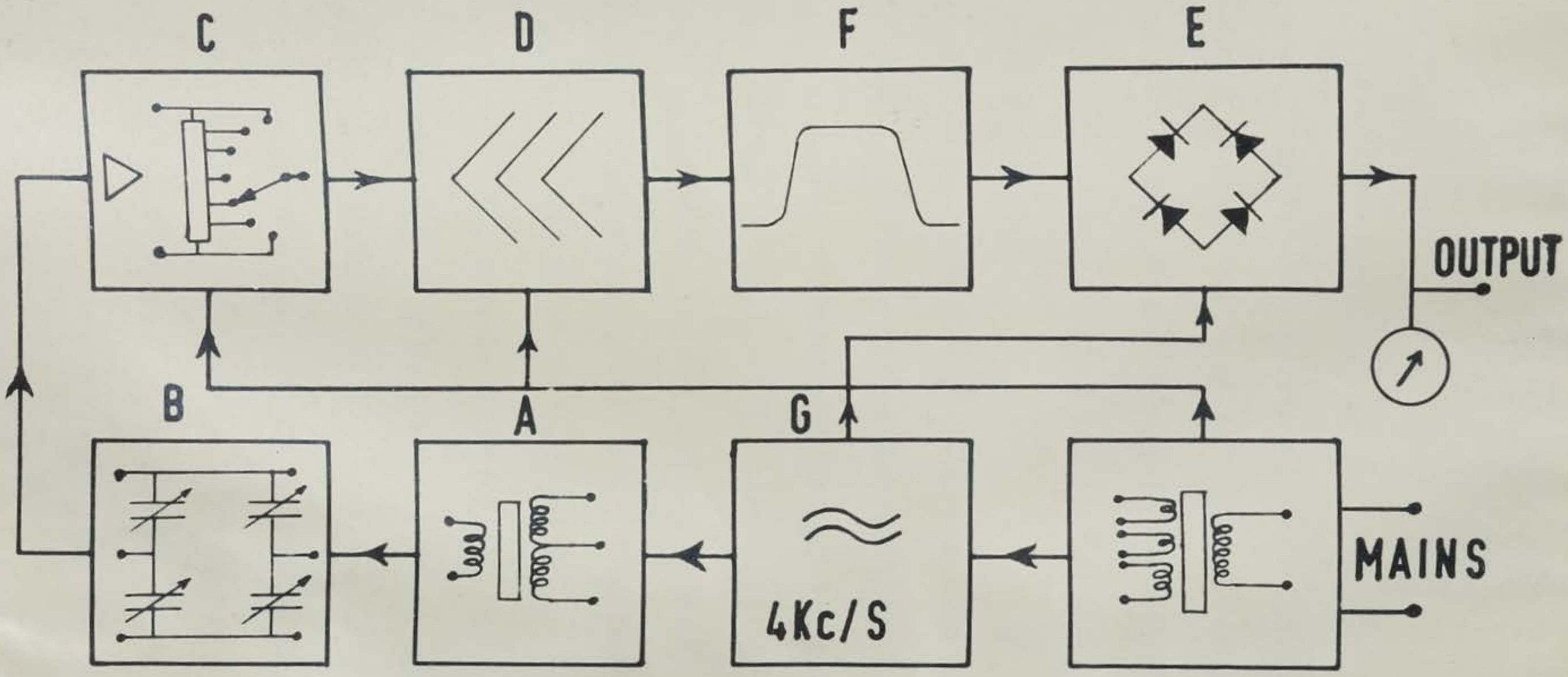


Fig. 17 BLOCK DIAGRAM of MEASURING BRIDGE

Operation.

This system, being exceptionally stable, required only occasional correction. Such correction was achieved by the alternate adjustment of the "phase" and "amplitude" knobs on the front of the instrument to obtain zero readings on the meter. Calibration was carried out in the same way as that for the original system.

In the later system an inductive pick-up was used to measure the deflection of the diaphragm (figure 18). The pick-up unit B was mounted on a sturdy bracket protruding from the main column of the machine. The deflection of the diaphragm A was communicated to the pick-up by means of the lever C; this was pivotted at its centre to give a 1:1 displacement ratio. This lever terminated in an aluminium pressure pad H, with a hemispherical end, resting on the centre of the diaphragm to give a concentrated point of load application. The position of the pick-up was adjusted relative to the machine by the nut D so that the plunger E was at the centre of its travel. This then exerted a load on the lever of 20gm. which slightly pre-loaded the system and kept the pressure pad in contact with the diaphragm.

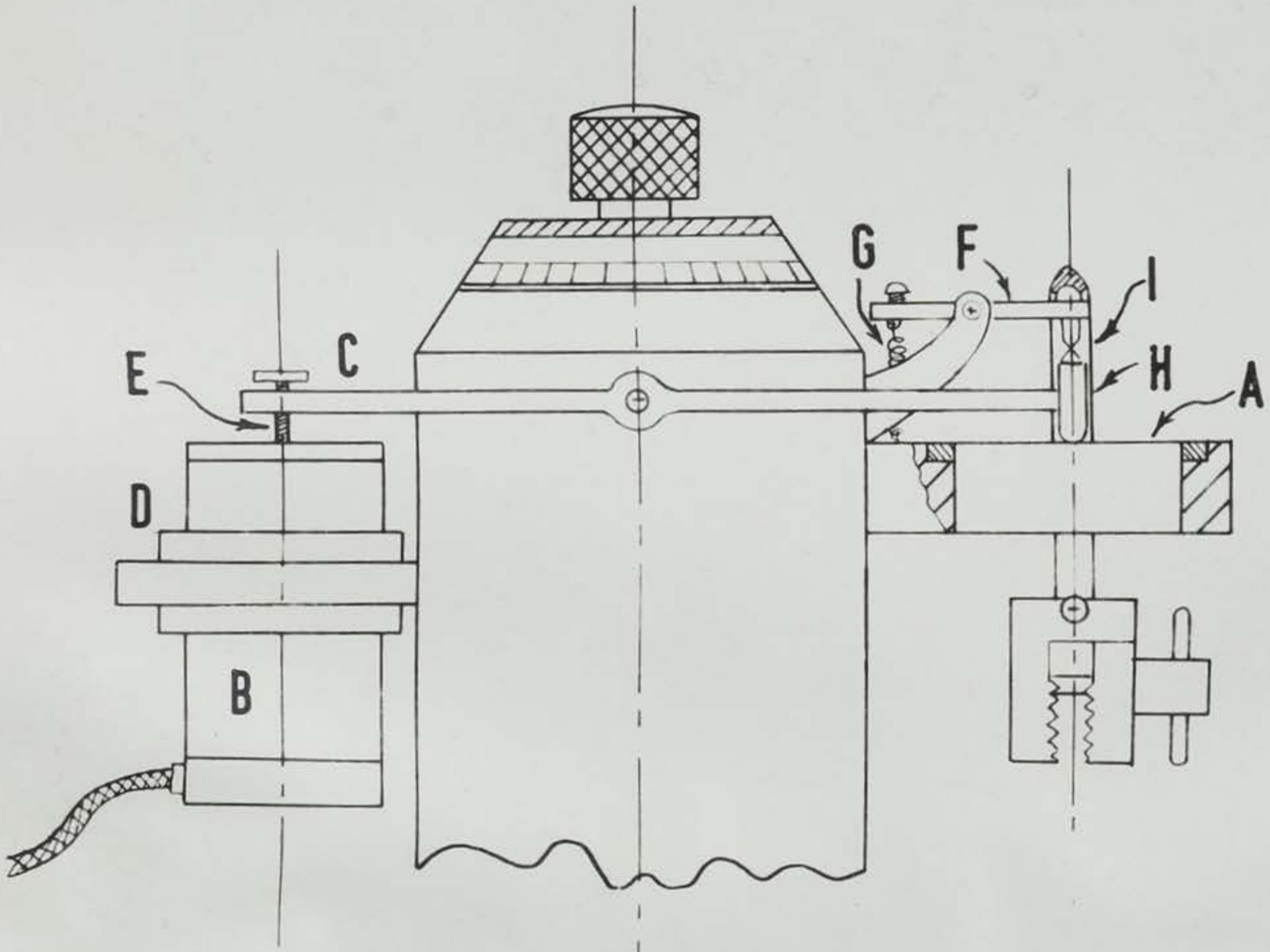


Fig. 18 LOAD SYSTEM

A second shorter lever F, also pivotted at its centre and electrically insulated from the rest of the machine, was mounted above lever C. The top jaw of the machine was suspended from a point on this lever directly over the pressure pad H. The weight of the jaw (20gm) was balanced by a force of 25gm. supplied by the spring G at the rear of the lever. In this way it was necessary to have a tension in the specimen of 5gm. before the levers came in contact; thus giving a load reading on the recorder and enabled a "standard zero" to be established.

On the top of the pressure-pad H and on a point of lever F immediately above it was mounted a small piece of platinum which in the unloaded state were held 0.5mm. apart by the spring. When a load was applied to the jaw the platinum contacts were drawn together and touched at 5gm. completing an electrical circuit fitted to an alarm mechanism, to give indication that this load had been reached and that the test could proceed.

In normal tests the recorder was calibrated and zeroed, as described, with the contacts open. The skin was extended by turning the machine manually, until warning was given by the alarm. At this point the machine was started and the test carried out.

Performance data.

Before the extensometer was used it was necessary to establish that it had linear characteristics with regard to load. Irregularities for example, could be due to the fact that diaphragm was over-loaded or that some of the electronic circuits were out of balance.

It is well known that a steel spring obeys Hooke's law (i.e. produces a linear load extension characteristic, providing that it is not strained beyond its elastic limit). A small precision spring was tested, which could safely be loaded to 2kg. The linearity of this spring was checked on the Instron extensometer, for which calibration curves are obtainable. It was found that within the range of 0 - 1500 grams the spring had a linear response to within 0.25% of the maximum load and could therefore for all practical purposes be considered as giving a linear response. The spring was then tested again on the skin extensometer. The load measured by the extensometer was $2\frac{1}{2}\%$ high at the centre of its range (figure 19). This error was small and a calibration curve was constructed to correct results. The adjusted values used are shown in the Table 1. The performance of the machine was checked periodically against a standard curve to ensure that this standard of performance was maintained. As an additional check, each time before use

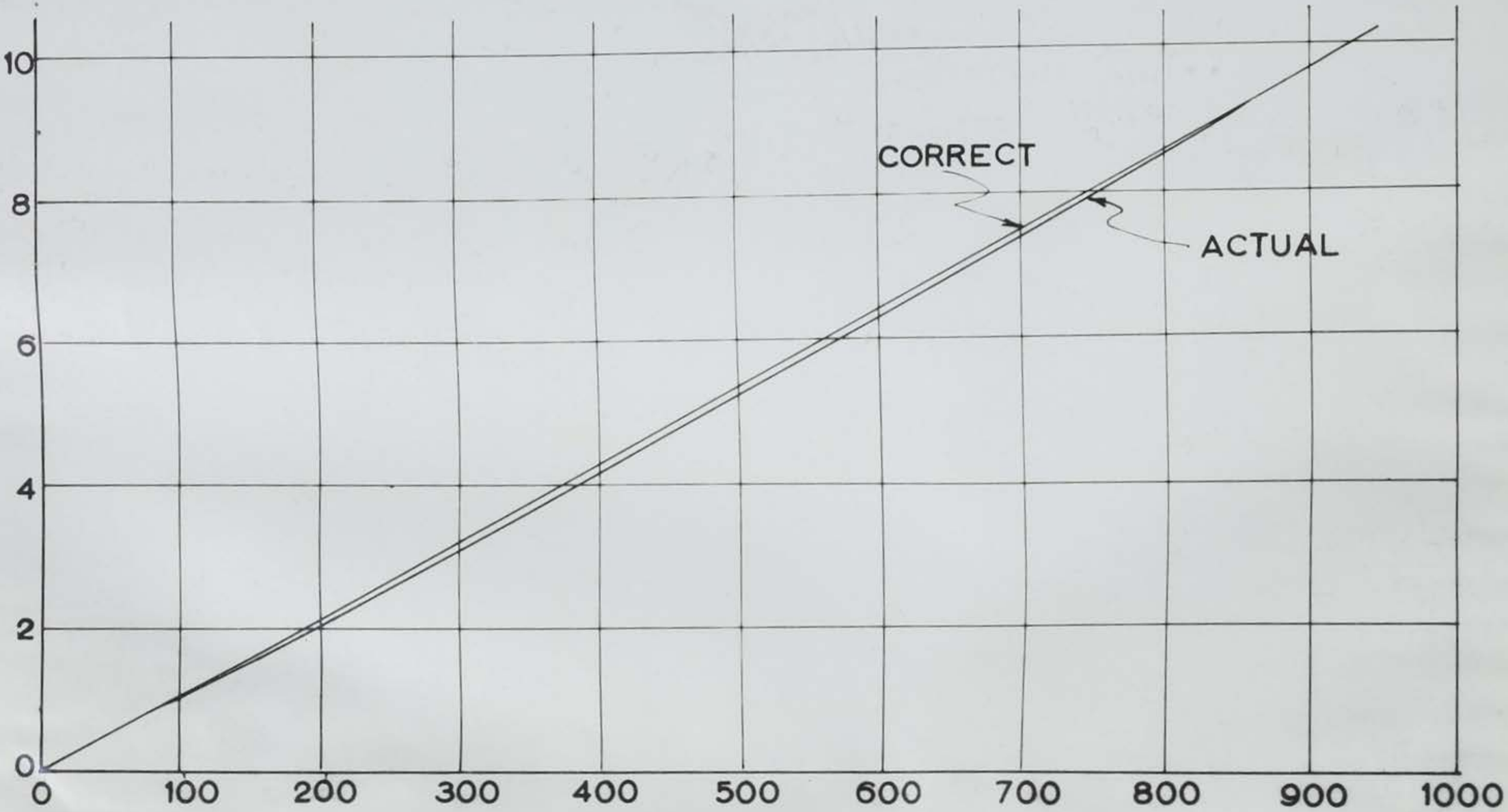


FIG. 19

CALIBRATION OF EXTENSOMETER WITH STEEL SPRING

the machine was calibrated with a known mass suspended from the top jaw.

LOAD AS READ	ACTUAL LOAD GRAMS
100	100
150	148
200	194
250	244
300	293
350	342
400	391
450	438
500	488
550	540
600	590
650	640
700	692
750	742
800	792
850	843
900	895
950	948
1000	1000

Table 1. True and actual load values of a precision spring tested on the skin extensometer.

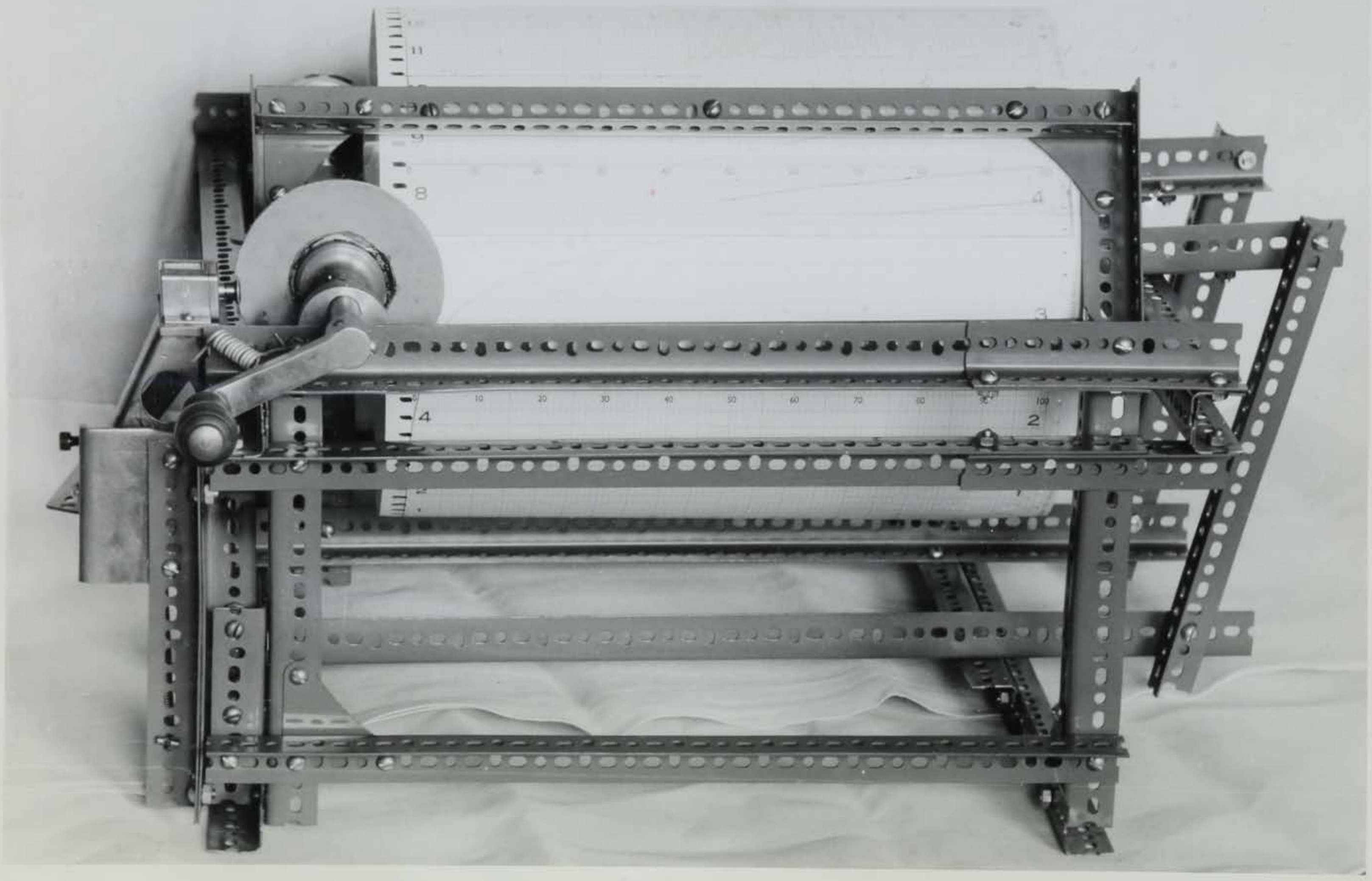
C. MEASUREMENT OF STRESS STRAIN CURVES

To simplify the measurement of the 19 ordinates used in the curve fitting technique described later, a machine was developed (plate 14). This is shown diagramatically in figure 20.

A drum A, 9 inches diameter, was mounted axially in a "Dexion" frame on conical pivots B1 and B2 with a worm-wheel C attached to its shaft. A second shaft D which ran perpendicular to the axis of the drum carried a worm which engaged with the worm-wheel. Pivot B1 was spring loaded in such a way that the worm-wheel was pressed into mesh with the worm. This also prevented back-lash and enabled the gears to be pushed out of mesh so that the drum could revolve freely for setting up purposes.

The shaft D which ran in brass bearings was fitted with a friction disc E which was faced with rubber and a handle H to enable the shaft to be rotated manually. A revolution counter F, which had had its first digit ring locked to the counter input shaft to enable tenths of a revolution to be recorded, was fitted with a wheel I, half an inch diameter. This was mounted on a spring bracket and held with wheel I in contact with the friction disc by means of a spring G. It was possible to move the counter in the direction X ↔ X so that the effective radius of the

PLATE 14. Ordinate measuring device.



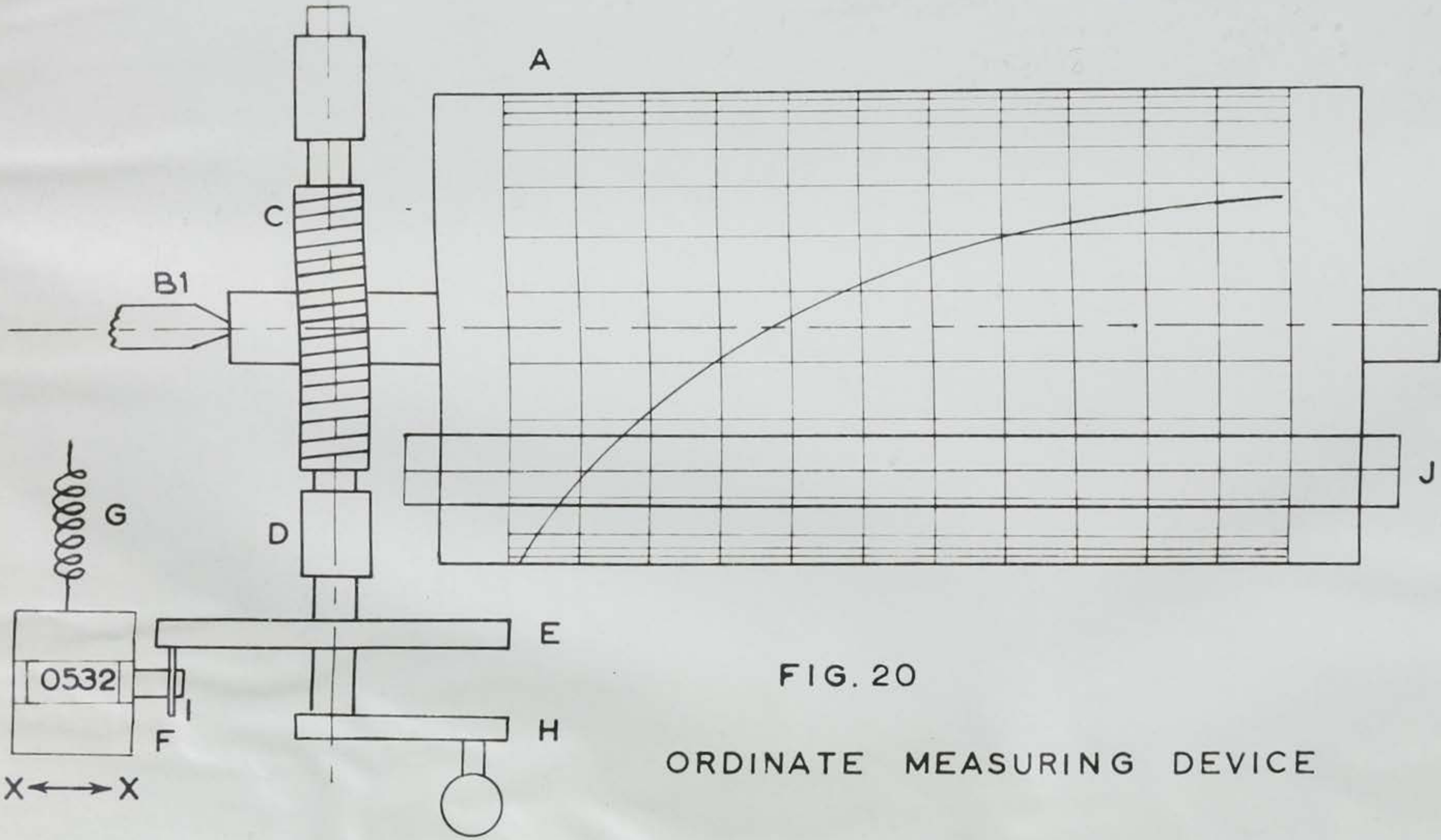


FIG. 20

ORDINATE MEASURING DEVICE

friction disc could be altered to change the ratio of the system for adjustment purposes.

As the worm, worm-wheel ratio was 60:1 when the friction drive ratio was adjusted to 6.37:1, one digit on the counter corresponded to a movement at the surface of the drum of 0.003 inches. When a stress strain chart was wrapped round the drum this represented an extension of 0.0001 inches (chart magnified extension thirty times).

A perspex sheet J with a fine black line engraved in it was mounted against the surface of the drum. When the chart was mounted with a selected datum coinciding with the engraved line, rotation of the handle caused the chart to move until a section of the curve passed the line and the length of the corresponding ordinate was read directly from the counter.

D. DETERMINATION OF PERCENTAGE OF COLLAGEN IN SKIN

1. SKIN PREPARATION

Prior to determining the amount of collagen the dry weight of the specimen was measured. It was found that by drying in an oven, a steady state could not be achieved even after 100 hours at 60° C. - higher temperatures could not be used because of the thermal shrinkage of collagen at 65° C. (Akeson, 1963). This was due to the fat content of the skin, even though all the loose fat had been removed. Various solvents were used to defat the skin before drying but they failed to have the necessary penetration. Mincing and grinding were used to break down the skin but these proved unsatisfactory owing to the size of the specimen.

A successful method was finally evolved in which the specimen was placed in liquid nitrogen, contained in a large Dewar flask and cooled to nearly -200° C. On removal the skin was "glass hard" and in a form which could readily be crushed by means of a crusher specially developed to prevent shattering and consequent loss of material.

Skin crusher.

The crusher was made of stainless steel throughout to the design shown in figure 21 and plate 15. It consisted of three main parts:- a collarB, a plunger A and an anvil C.

PLATE 15. Frozen skin crusher with the collar, plunger, anvil, retaining pin and light springs separated.

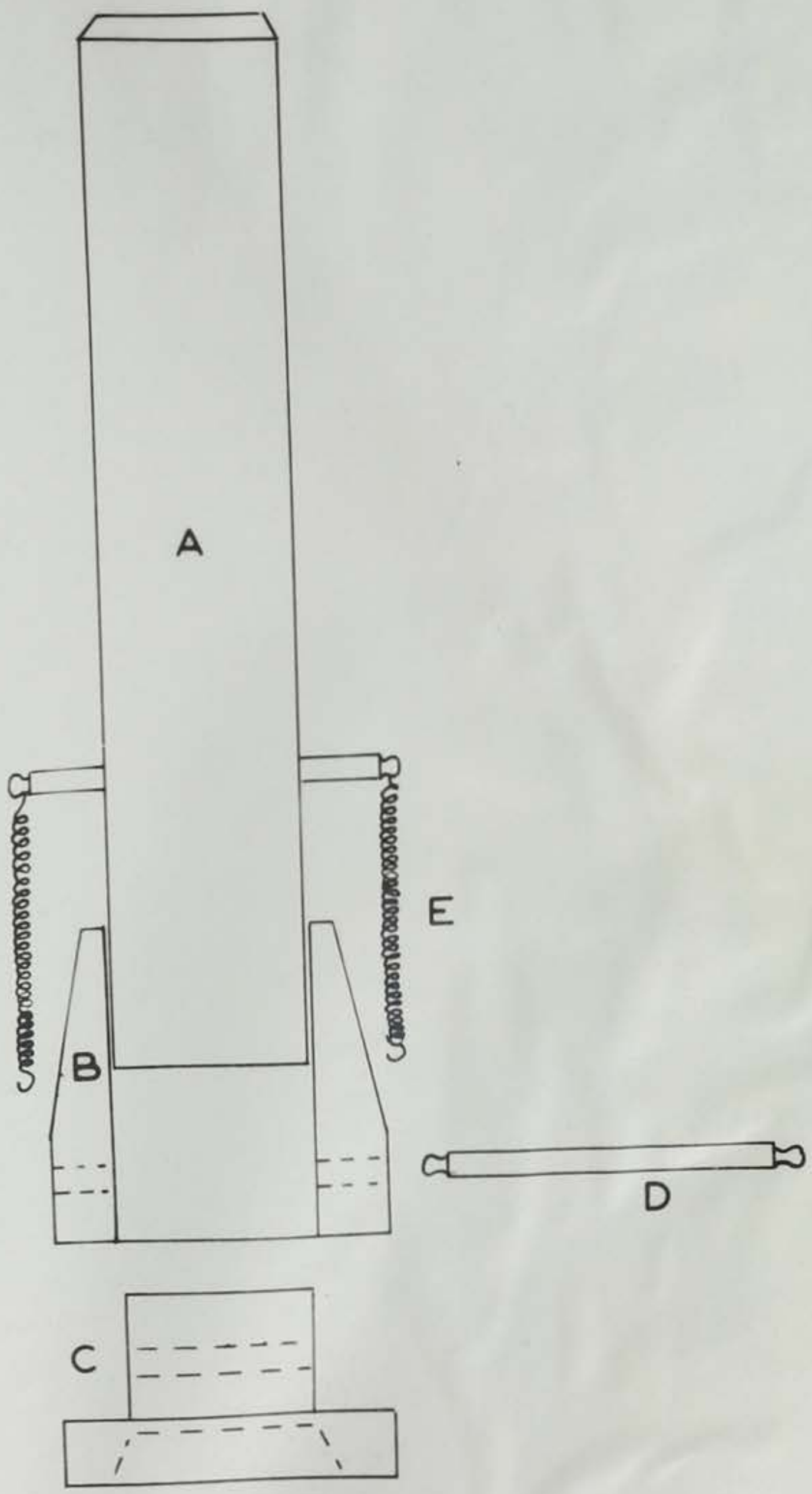
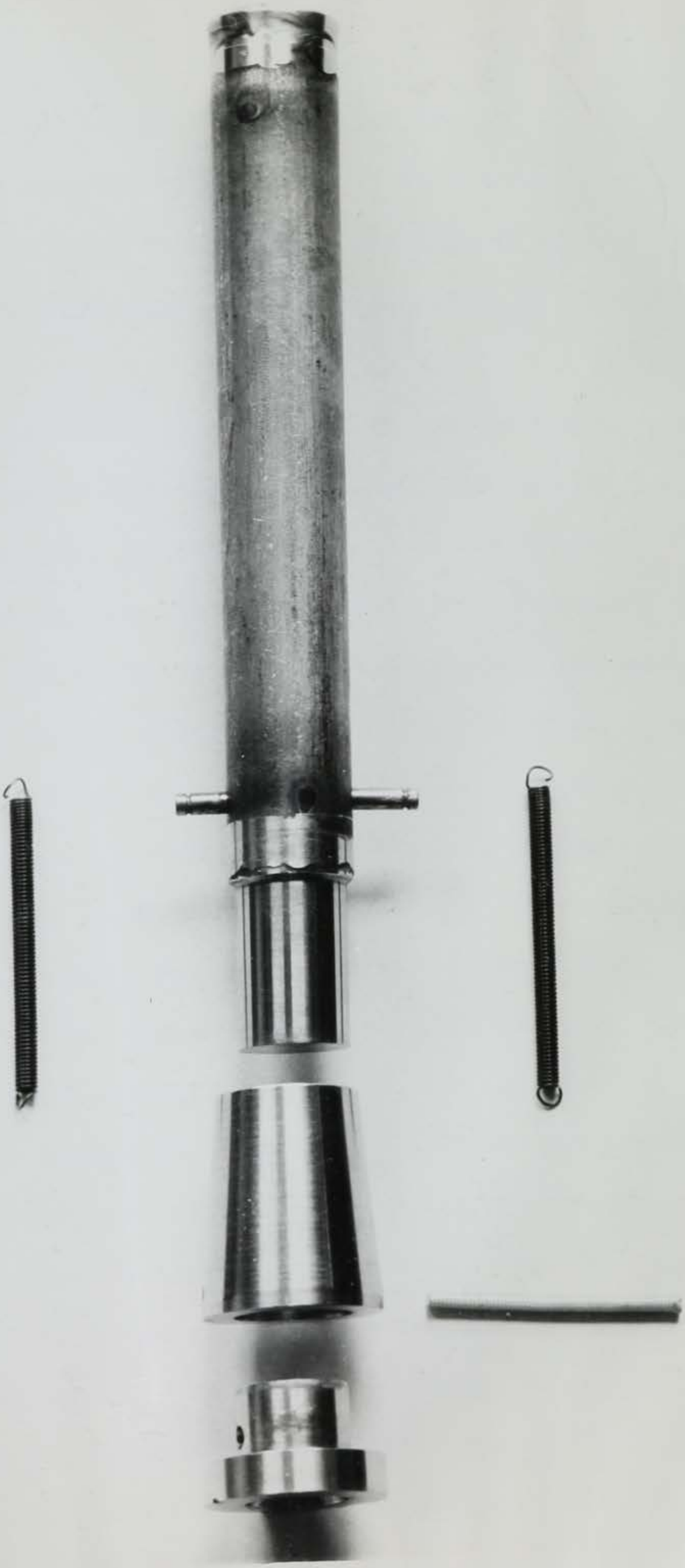


FIG. 21

FROZEN SKIN CRUSHER



The plunger and the anvil fitted into the collar, the anvil being retained by the pin D. It was found necessary to have a 0.006" clearance in the bore of the collar to prevent seizure when shrinkage took place in the nitrogen.

To reduce the heat equivalent of the crusher in order to conserve nitrogen, the plunger was made of a tube with capped ends, and the anvil had a recessed base.

The crusher was used as follows:- the anvil was secured in the collar by means of a pin, and a specimen of skin placed in the collar and the plunger inserted. The two light springs E were hooked on the pin D to hold the parts together. It was found advantageous to pre-cool the crusher by standing it in the nitrogen before placing a specimen in it. This cause the skin to be lightly frozen before the plunger was inserted, so that the pressure of the springs would not flatten the skin and prevent the optimum crushing effect (this was not necessary after the first specimen had been cut, as the crusher was still cold from its previous immersion). When the crusher was assembled, it was placed in the nitrogen until all boiling had ceased. On removal it was placed on a solid metal base and the specimen forcibly compressed. This reduced the skin to a finely divided form.

Water and fat were then removed by leaving in a test tube of acetone overnight. The following morning the acetone was changed and the tubes shaken vigorously for 15 minutes in a mechanical shaker, after which the acetone was changed and the tubes again shaken.

2. BIOCHEMICAL METHOD

The percentage of collagen in the specimens was determined by a modification of the Neuman and Logan method (1950) introduced by Woessner (1961). It dependend on determination of percentage of hydroxyproline in the specimen.

Reagents.

Hydroxyproline standard.

A stock solution was prepared by dissolving 25mg. of L - hydroxyproline in 250ml. of 0.001N HCl.

Buffer.

Citric acid monohydrate 50gm.

Glacial acetic acid 12ml.

Sodium acetate trihydrate 120gm.

Sodium hydroxide 34gm.

Distilled water to make up 1000ml.

The pH was carefully adjusted to 6.0 and the buffer was stored in the refrigerator under toluene.

Perchloric acid.

27ml. of 72% perchloric acid diluted to 100ml.

with distilled water.

Methyl cellosolve.

2 - methoxy-ethanol

The following were prepared shortly before use:-

Chloramine T.

1.41gm. chloramine T was dissolved in 20ml. water, 30ml. methyl cellosolve was added along with 50ml. buffer. The preparation was kept in a glass stoppered flask.

P-Dimethylaminobenzaldehyde.

A 20% solution was prepared by adding methyl cello-solve to 20gm. of P-dimethylaminobenzaldehyde to a final volume of 100ml. This could be warmed to 60° C. in a water bath to facilitate solubalisation.

Method.

Prior to crushing specimens were removed from the storage bottles of frozen saline and placed on filter paper to remove the surplus water. The wet weight was deter-mined. The dry weight was estimated as described previously.

Portions of dried, de-fatted skin were weighed out to as near 10mg. as possible and the exact weight noted.

Each specimen was placed in a small test tube and 1ml. of 6N HCl added. The tubes were then sealed and placed in an oven at 105° C. for 48 hours, after which the tubes were opened and the contents decanted into a measuring cylinder. The tubes were washed thoroughly with distilled water and the washings added to the hydrolysate. A few drops of 0.02% methyl red were added and then the amount of 2N NaOH calculated to neutralise 1ml. of 6N HCl. The pH was carefully adjusted with dilute HCl and NaOH until the indicator turned slightly yellow, showing a pH value of 6.7. Distilled water was then added to make up a volume of 25ml. 1ml. of this preparation was taken and transferred to a volumetric flask and made up to 25ml. with distilled water. For the test 2ml. of this dilution was placed in a test tube.

A series of standards containing 0 to 5 µgm / 2ml. were prepared from the stock solution by diluting with distilled water.

Hydroxyproline oxidation was initiated by adding 1ml. chloromine T to each tube in a predetermined sequence. The tube contents were mixed by shaking a few times and then allowed to stand for 20 minutes at room temperature. The chloromine T was then destroyed by adding 1ml. of perchloric acid to each tube in the same order as before.

The contents were mixed and allowed to stand for 5 minutes and finally 1ml. of P-dimethylaminobenzaldehyde solution was added and the mixture shaken until no schlieren was visible. The tubes were placed in a water bath at 60° C. for 20 minutes and then cooled in tap water for 5 minutes. The developed colour thus formed was stable for at least 1 hour. The light absorbency of the solution in the tubes, which was proportional to the concentration of hydroxyproline in the original specimens, was measured using a spectro-photometer at a wave length of 557mμ. The concentrations of hydroxyproline were then determined directly from the calibration curve drawn from the standard values.

Calculations.

Dry skin contains approximately 70% collagen dry weight (Rothman, 1953) and collagen is known to contain 15% hydroxyproline (Bowes et al, 1955):-

$$10\text{mg. of skin contains } \frac{15 \times 70 \times 1000 \times 10}{100 \times 100}$$

$$= 1050\ \mu\text{gm. hydroxyproline approx.}$$

After neutralisation the hydrolysate was diluted to 625ml., to obtain a 2ml. sample containing 3-4 μgm. hydroxyproline in order that the optimum section of the calibration curves could be used. The hydroxyproline

concentration was approximately 3.36 µgm / 2ml.

Standards.

The stock standard solution contained 10mg. of hydroxyproline per 100ml., that is 100 µgm/ml. Thus if 2.5ml. of this was diluted to 100ml. the solution contained 5 µgm/2ml. and standards were made up accordingly.

Accuracy test of method.

In order to check the accuracy of the method, a specimen of gelatin, which had been accurately assayed in the Leather Department of Leeds University by Mr. C.H. Pearson, using the method of Leach (1960), was obtained and tested.

Approximately 1gm. of gelatin crystals were placed in a $2\frac{1}{2}$ inch platinum crucible and sufficient warm distilled water added to dissolve them. The crucible was then placed in an oven at 105° C. and a thin film of gelatin (which could be dried more readily than the crystals) was produced on the bottom of the crucible. It was dried to constant weight and the percentage hydroxyproline determined.

When the test had been completed the ash content of the gelatin was determined in order that the percentage hydroxyproline could be given on a dry ash-free basis.

The assay of gelatin given with the sample from the laboratory of its source was 14.4% hydroxyproline. The

value derived by the above experiment was 14.0%, which, when differences of experimental technique are taken into account, is an acceptable result.

General experimental procedure.

A number of specimens of skin were frozen, crushed and de-fatted in acetone. After drying at 55° C. these were placed in stoppered test tubes and kept until required. Each specimen was tested in duplicate. It was found that the optimum number of specimens which could be treated satisfactorily at once was five, giving twelve tubes in all, including the two standards.

V. RESULTS

A. GENERAL RHEOLOGICAL PROPERTIES OF SKIN

1. Normal stress strain curve.

It was found that if a specimen of skin was extended at a constant rate in the extensometer, a curve of the form shown in figure 22 was produced, where the vertical axis represents the extension and the horizontal the load applied. It is seen that initially a considerable extension was produced by a small load, but as the process continued the skin became more resistant, requiring a considerable increase in load to produce further extension. This was the reverse of what was found for tests carried out on strips of the plastic, polythene (figure 23), which is seen to be initially stiff, becoming more extensible at higher loads.

The extension of skin has been found to fall into two clearly defined modes, described by different mathematical equations. The initial stage corresponding to the fibre orientation obeyed the law

$$E = a + b \log L$$

where E = extension
L = load
a & b = constants

The second stage corresponds to fibre extension and is described by the equation

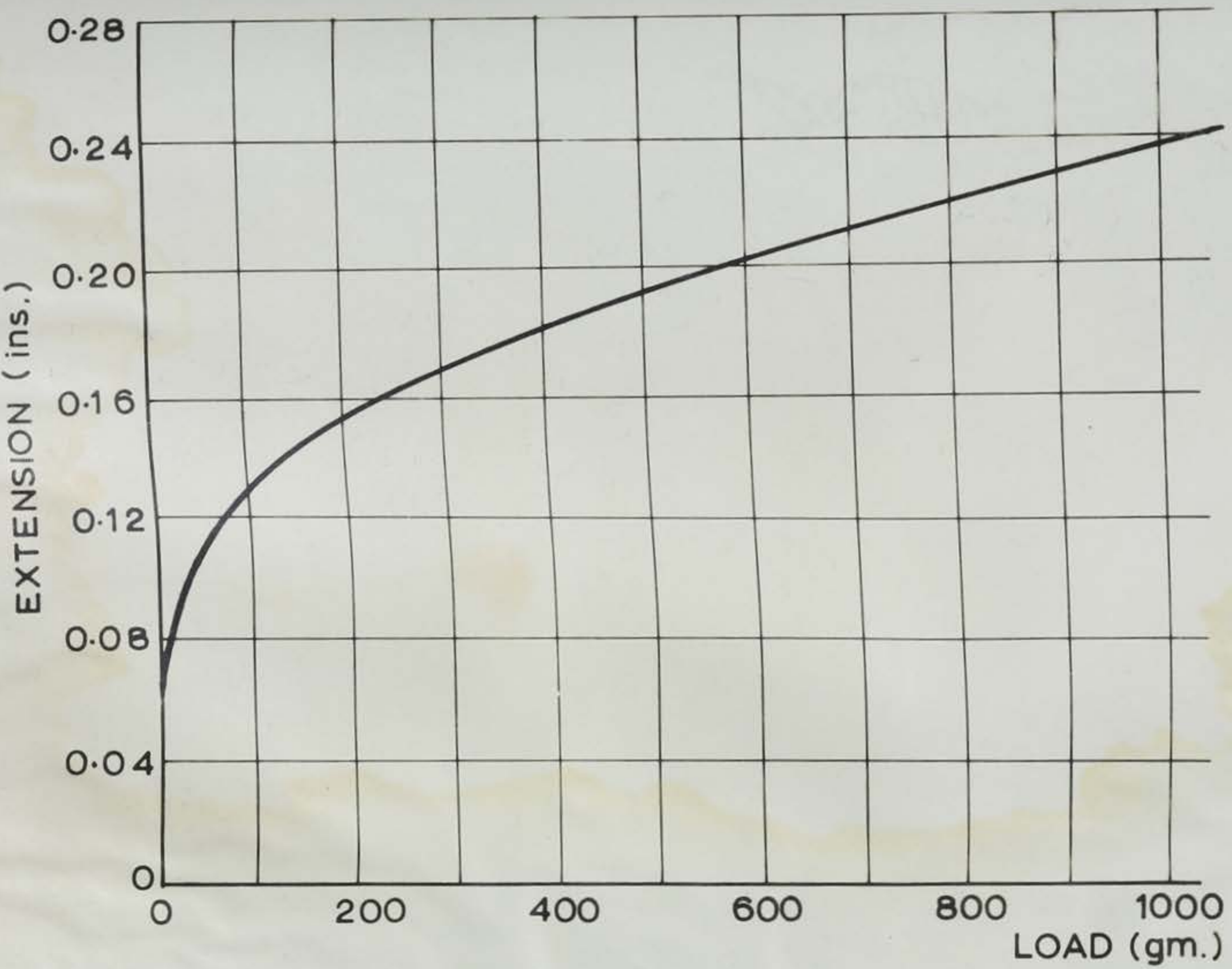


FIG. 22
CURVE FOR SKIN

FIG. 23
CURVE FOR POLYTHENE

$$E = c + k L^{b}$$

where E = extension
L = load
c, k & b = constants

The mathematical significance of these equations is discussed in detail later (section V.B).

2. Yielding.

If the extending process was continued, the specimen eventually yielded and the stress strain plot was of the form shown in figure 24. It is seen that the line gradually became steeper and then eventually turned and began to return to the extension axis as the load fell off. This is of interest, because it demonstrated that the specimen did not rupture suddenly but underwent a gradual yielding process, suggesting that at some point the individual fibres began to slip over one another, probably due to the breakdown of friction or bonds between them. Specimens were usually found to yield about their mid-point in the load range 1,000 to 2,000 grams. In a few exceptional cases yielding occurred considerably below this and was found to be due to severe local damage to specimens by the jaws of the machine. This effect was reduced by the adoption of the dumbell shaped specimen.

3. Permanent set.

If a specimen was tested in the normal way to a point considerably before yielding and the extending mechanism

EXP. 573

MALE ABDOMEN
2 DAYS OLD

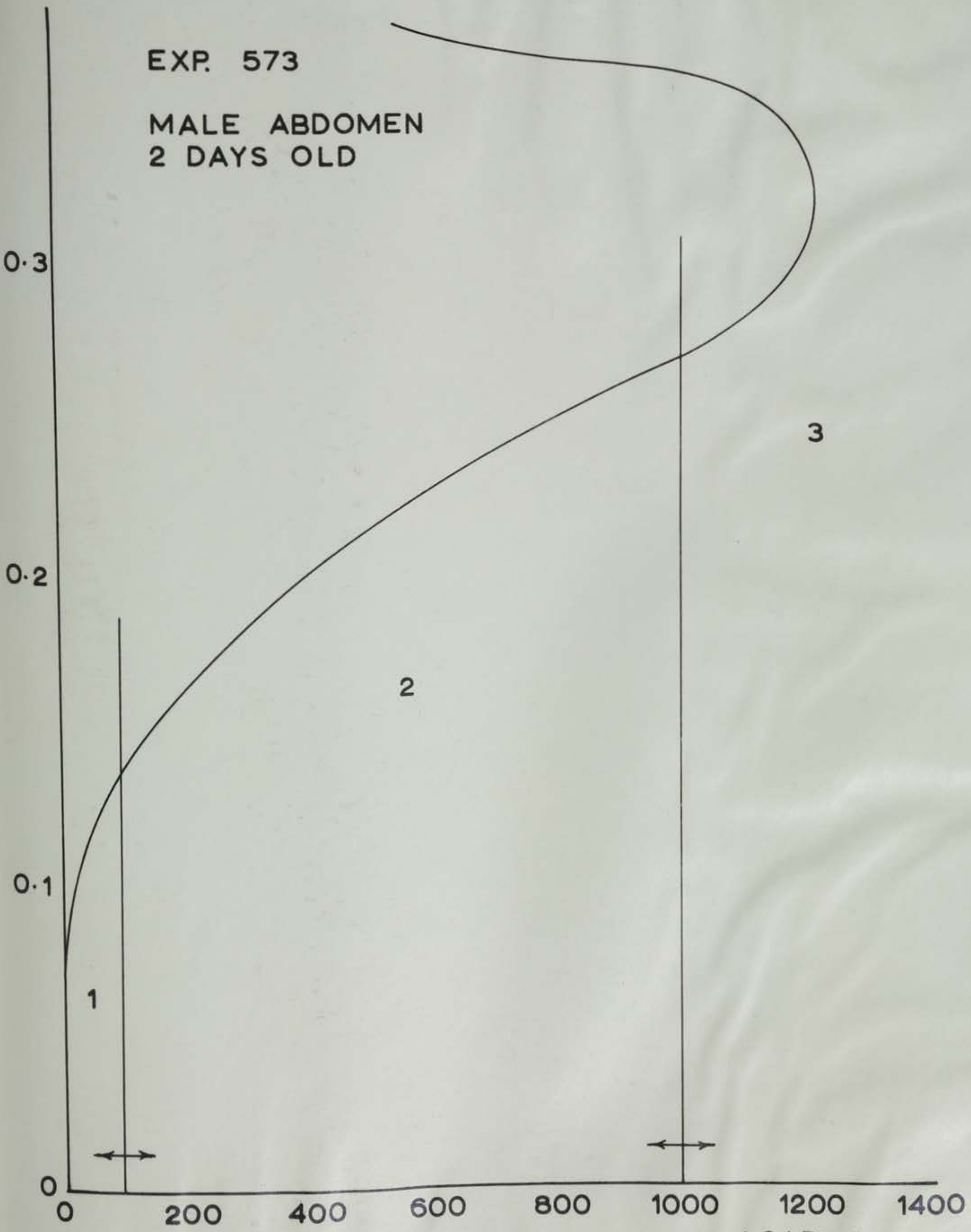


FIG. 24

LOAD/EXTENSION CURVE SHOWING THREE STAGES

reversed so that the jaws of the machine were brought together again at a constant rate, a graph of the form shown in figure 25 was produced. The section of the curve A B represents the normal extension process. This was reversed slightly in excess of 200 grams, and the return curve B C was produced. It is seen that this was less steep than the extension curve, resulting from the fact that when the load had been returned to zero there was still an appreciable extension A C remaining. This phenomenon is known as permanent set and represents some form of irreversible change in the specimen.

4. Progressive load cycling.

When a piece of skin was subject to a small extension, e.g. 2.1mm. (point 1 on figure 26), and then the extensometer was reversed until the load came back to zero, a small amount of permanent set remained, as described above. When the specimen was extended again from this point of zero load to 3.8mm. (point 2 on figure 26) it is seen that when the extension was somewhat greater than on the first occasion its course was ultimately the same as that which would have been adopted by the first extension had it continued up to this point. This process could be repeated any number of times, for example to 5.3mm.

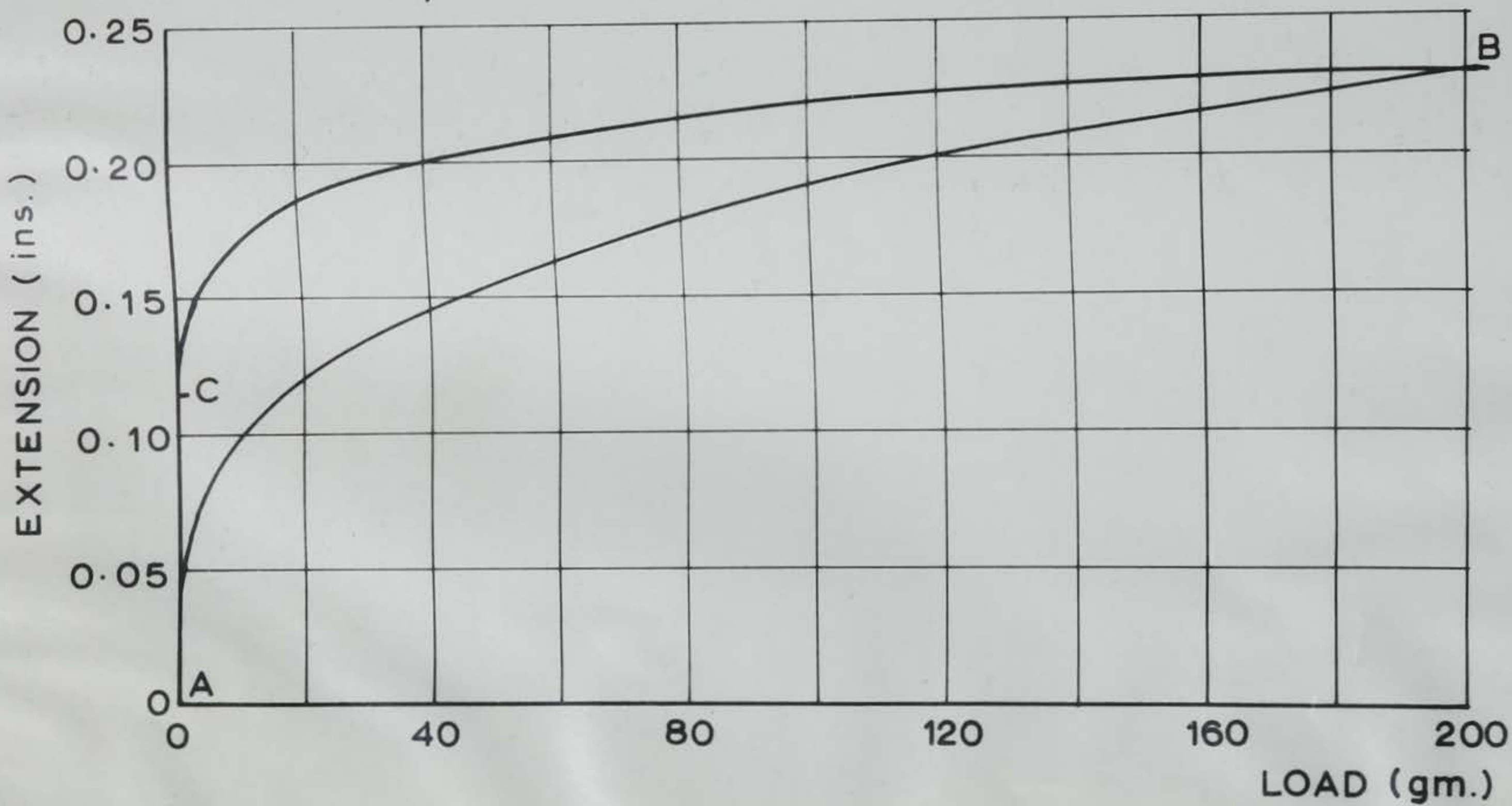


FIG. 25
PERMANENT SET

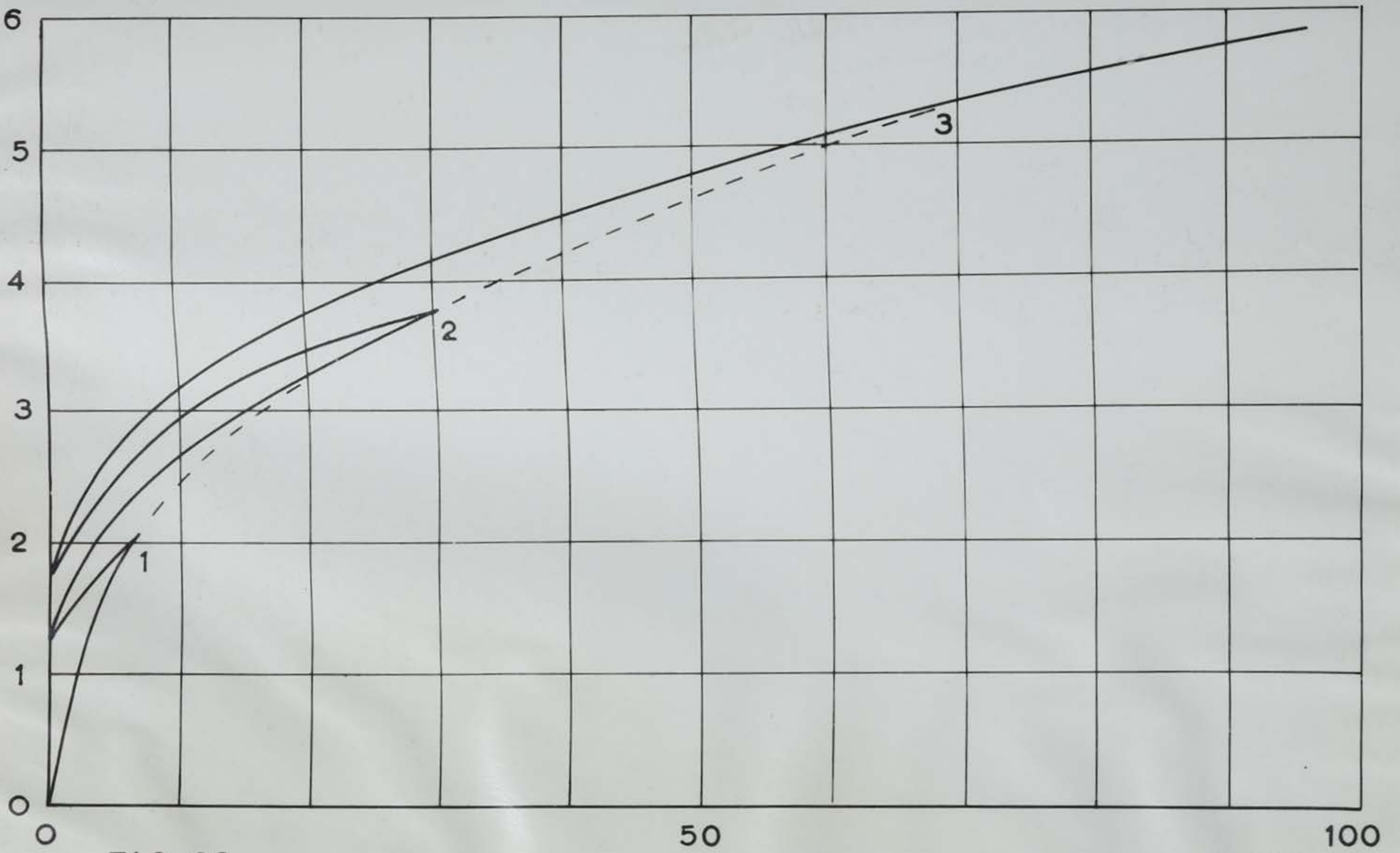


FIG. 26
PROGRESSIVE LOAD CYCLES

(point 3 on figure 26), provided that each successive extension was greater than the previous one. The fact that the composite curve built up from extension and relaxation cycles was the same as that which would have been adopted by a single initial large extension may be demonstrated by plotting the results of the experiment on semi-log paper (figure 27). It is seen that the second and third extension cycles produced curves which became tangential to the projection of the line produced for the first cycle. This has also been demonstrated for various textile fibres (Meredith, 1956 and 1958).

5. Progressive pulsatile loading.

Figure 28 shows the result of extending at a normal rate with the extending mechanism switched off for nine seconds in every ten. This was achieved by using a cam which operated a microswitch connected to the chart drive motor. It was shown that the shape of the curve was the same as that of a specimen extended in the normal way, demonstrating that the constants in the equation are purely dependent on the rate of extension, but independent of the manner in which the extension is carried out. Figure 29 shows a magnified portion of this stress strain curve obtained. The extension was stopped at point B and the

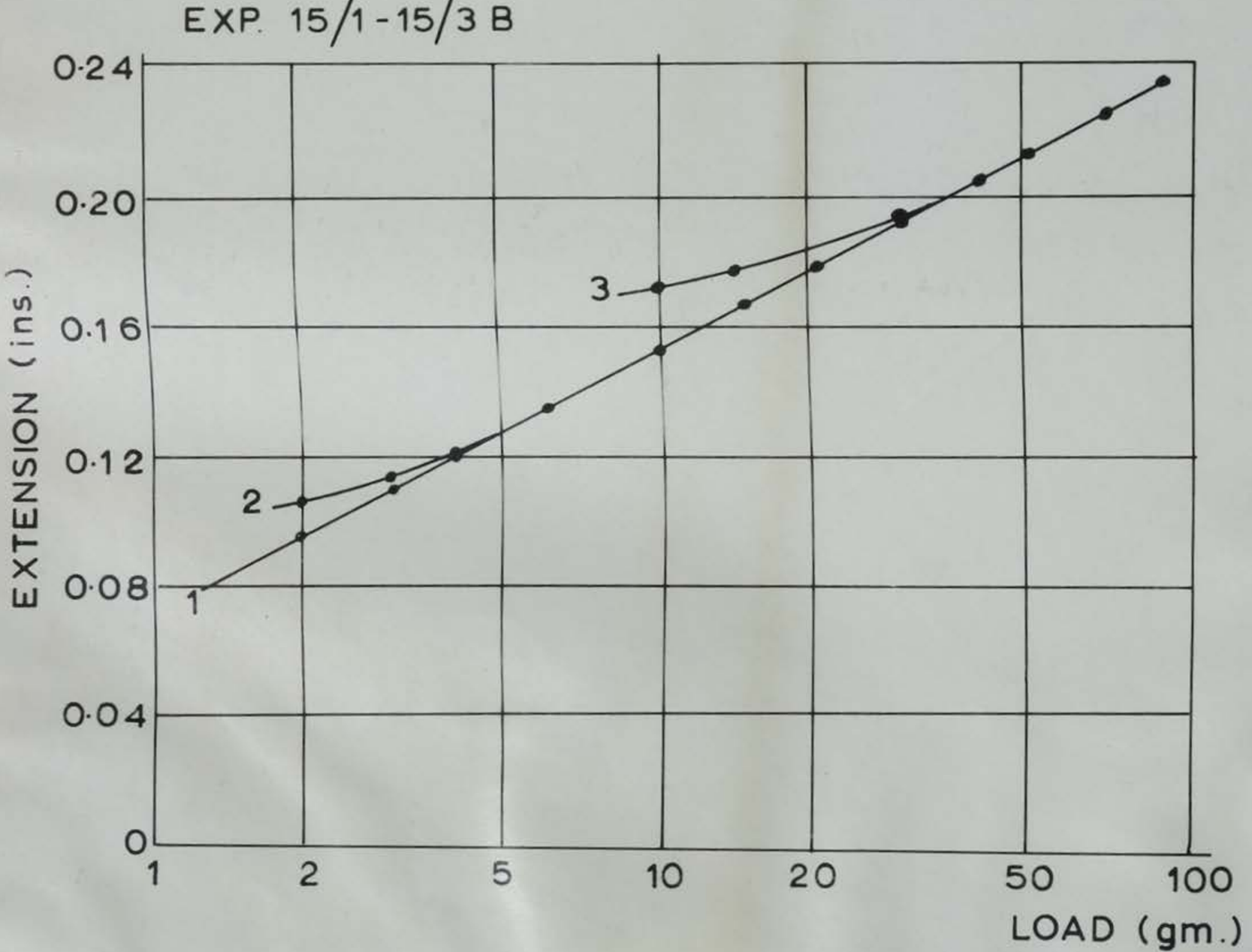


FIG. 27
LOAD CYCLING

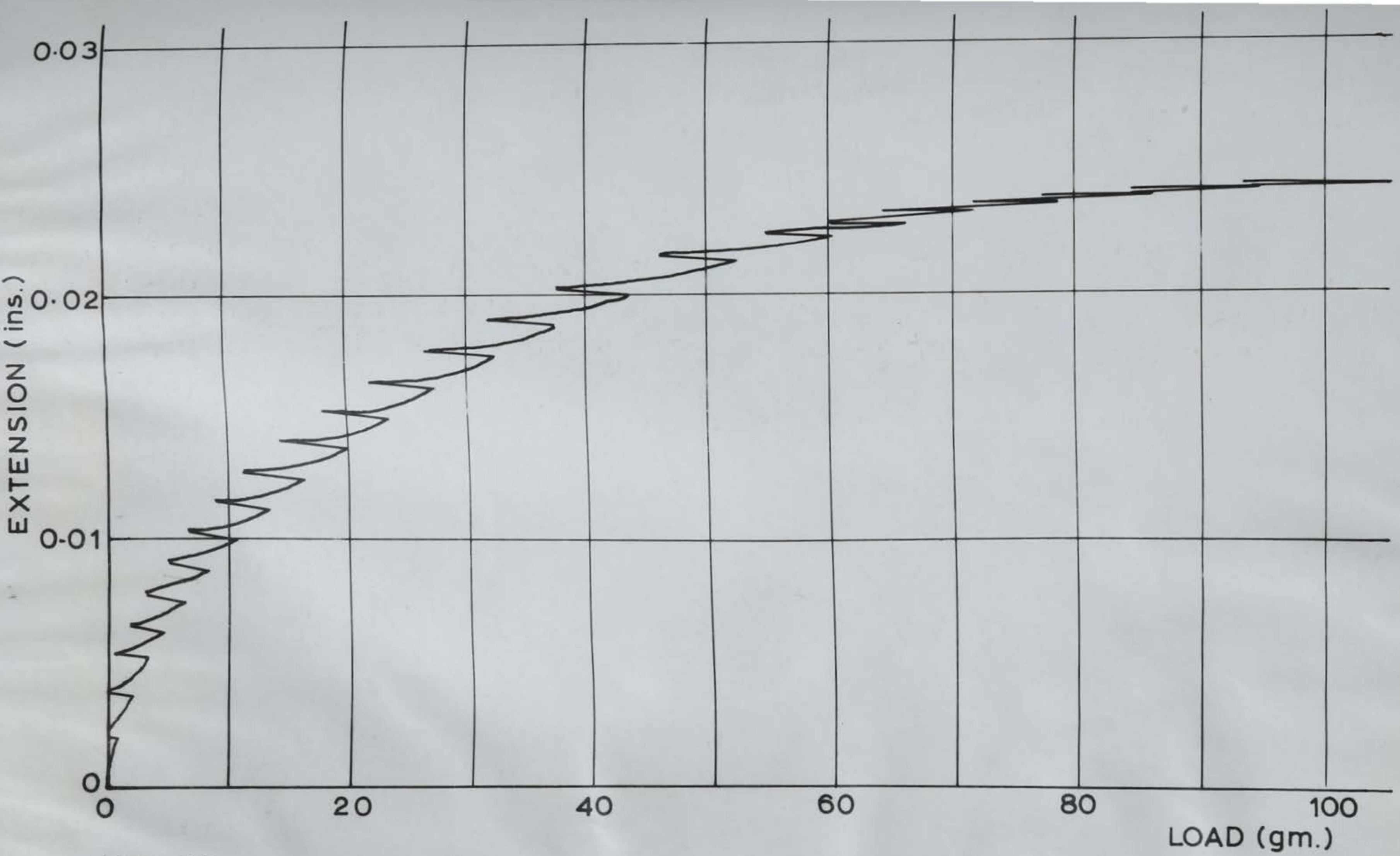


FIG. 28
PULSATILE LOADING

load relaxed to point C in nine seconds. The extenso-meter was then turned on for one second, and the curve C E was produced. It is seen that D E represented a further section of the major curve. C D however does not lie on the main curve, and is seen to have a lower gradient, indicating that during this period the specimen demonstrated greater stiffness.

6. Stress relaxation.

Figure 30 shows the effect of extending a specimen in the normal way to a value B. At this point, the magnetic clutch was turned off, so that extension ceased but the chart drive system continued to operate. In this way the stress relaxation curve B C was plotted as the load diminished with time. It is seen that initially the load decreased rapidly, but as time progressed it reached an almost steady value. The mathematical function characterising this curve was

$$E = c + d \log t$$

where E = extension
d & c = constants
t = time (secs)

7. Stress recovery.

Skin was found to demonstrate appreciable stress recovery. Figure 31 shows the curve for a piece of skin

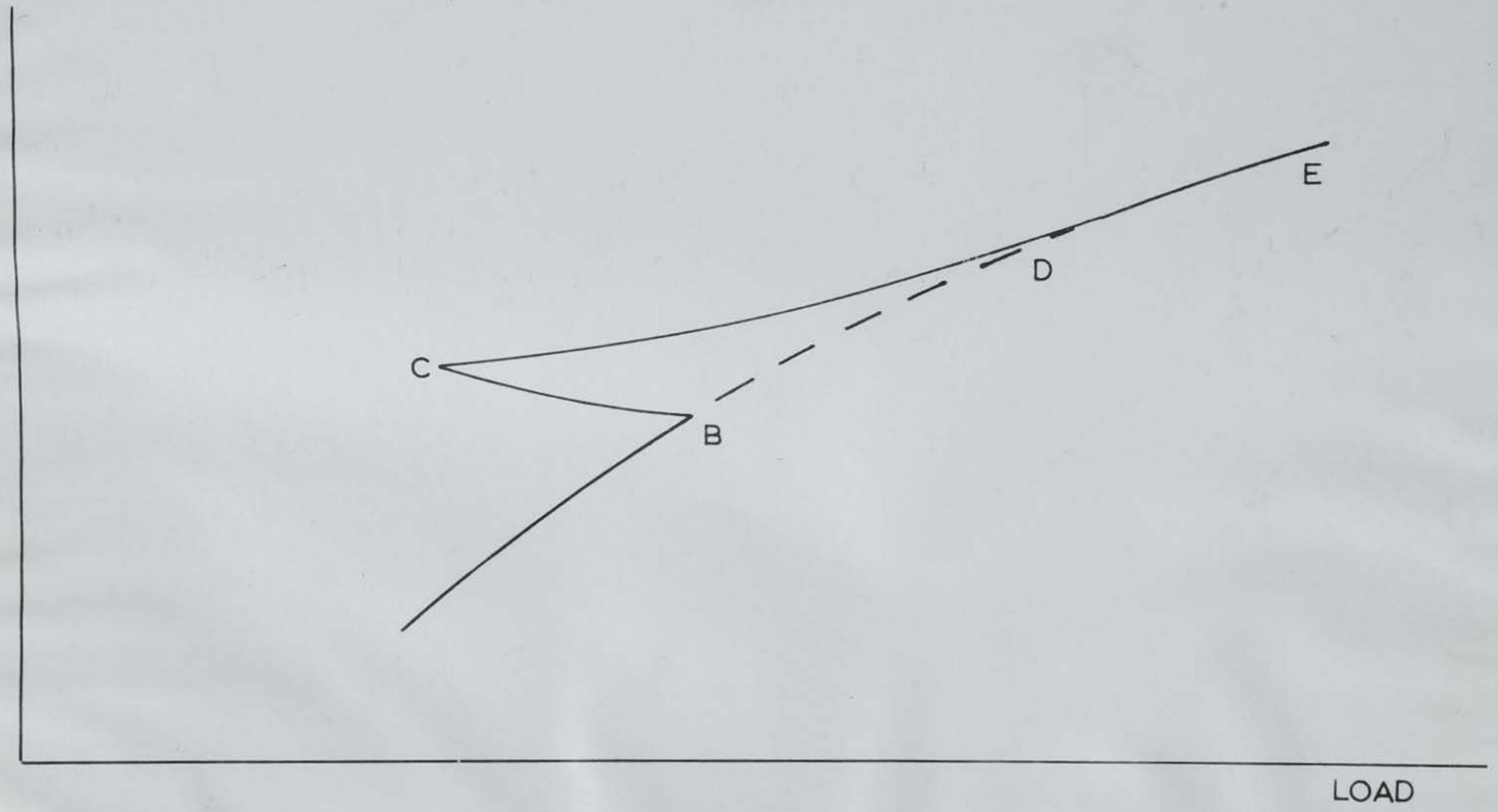


FIG. 29
MAGNIFIED PORTION OF PULSED CURVE

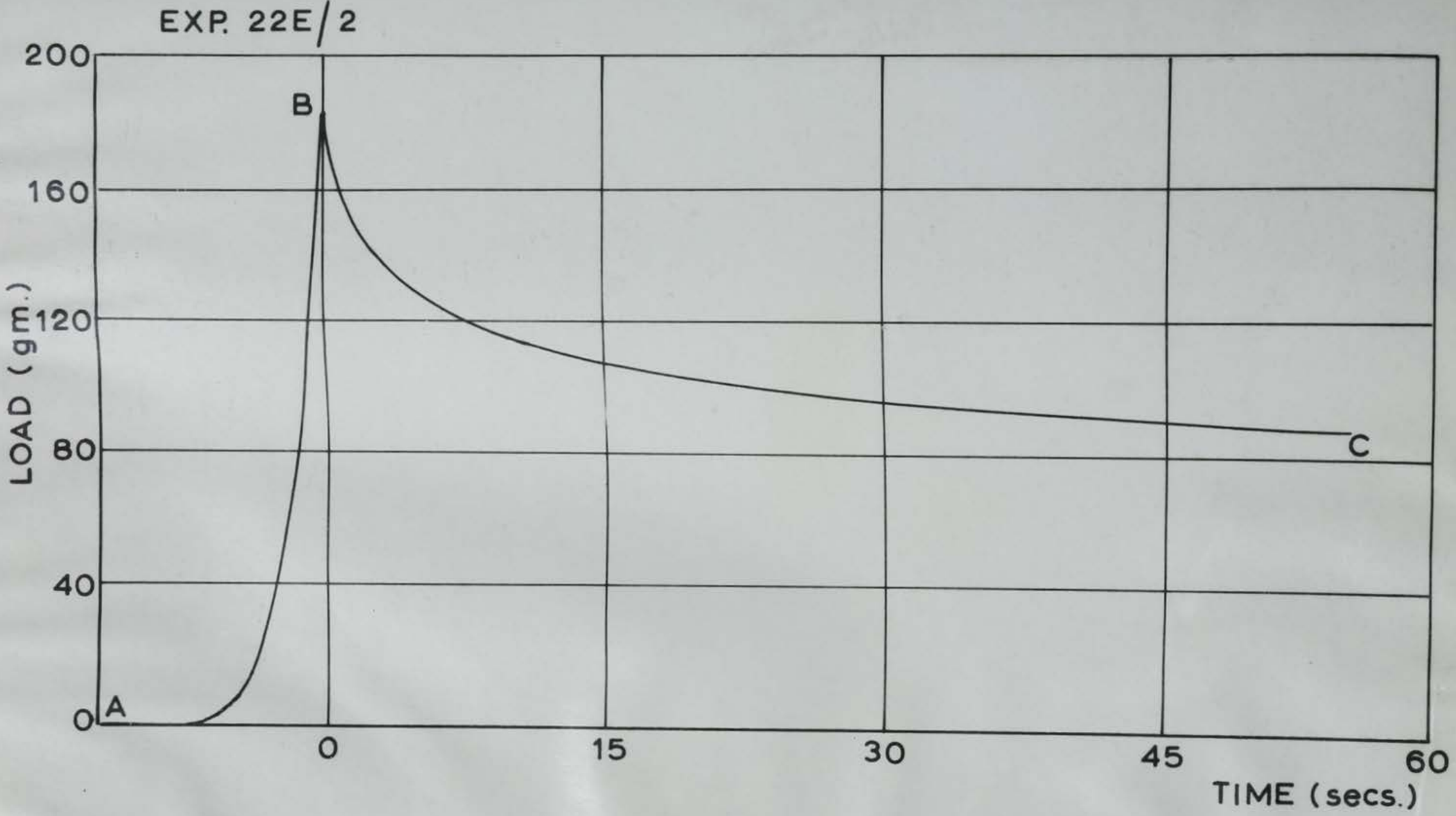


FIG. 30
STRESS RELAXATION

which had been subject to stress relaxation for 62 seconds. A sudden reduction of extension produced a fall in load from 75gm. to 39gm. and then recovery to 46gm. A further decrease in extension after a delay of 25 seconds resulted in a fall to 20gm. which recovered to 26gm. This is a property which is characteristic of many elastomers.

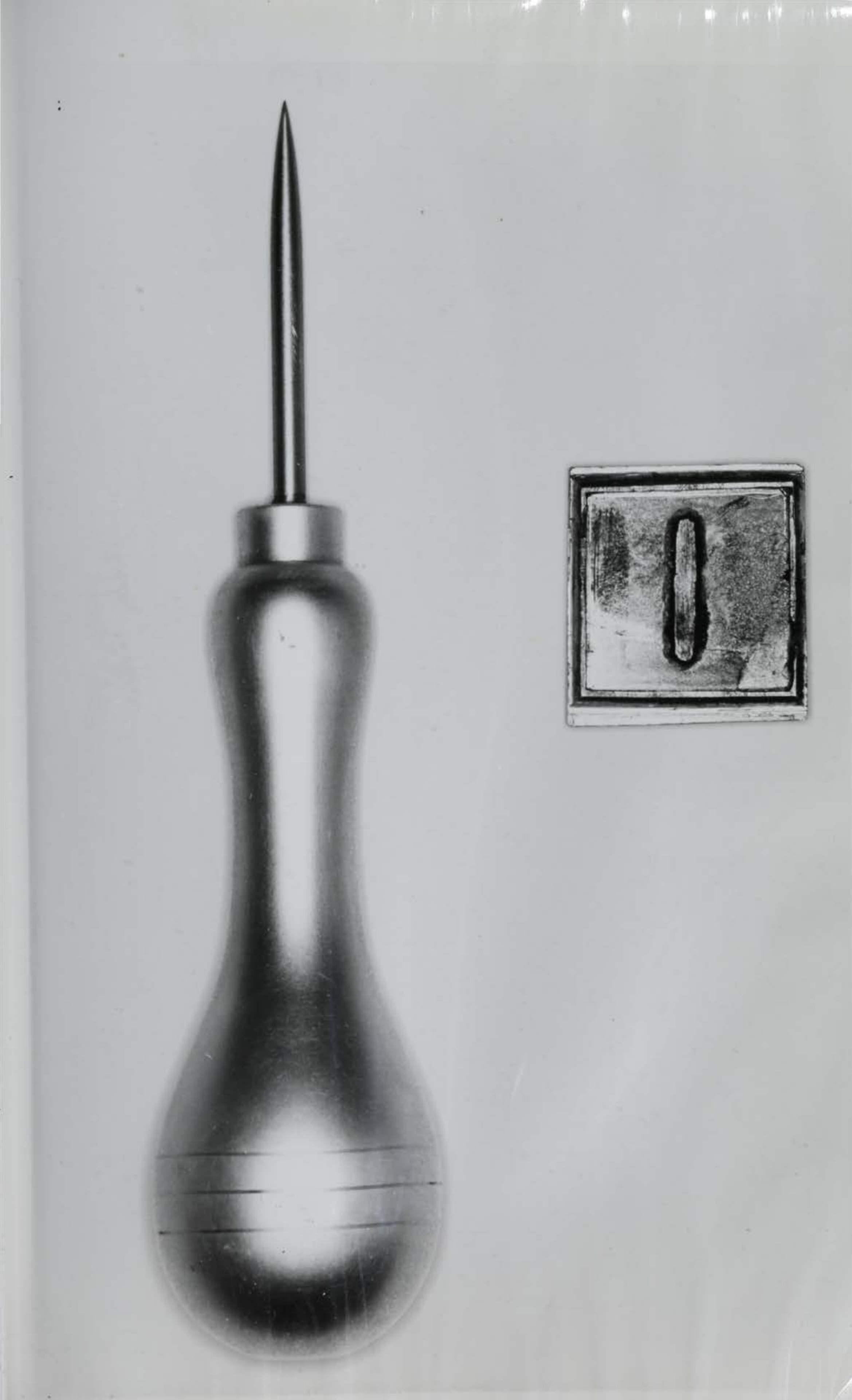
8. Rate of extension.

Tests were carried out to investigate the effect of changing the rate of extension of a specimen. A large piece of female abdominal skin was cut into 30 specimens. Half of these were mounted and tested at the normal rate of 0.2 inches per minute, while the other half were tested at 0.05 inches per minute. The specimens tested at the normal rate of extension gave an average value for b of 0.28 while those tested at the lower rate gave an average value of 0.20. It is thus seen that the value of b is highly dependent on the rate of extension and that the effect of lowering the rate of extension was to lower the value of b by 28.6%.

9. The directional effects of Langer's lines.

The direction of Langer's lines as stated by Cox (1942) was verified by use of an awl with a stainless steel point 3/16 inch diameter (plate 16). A number of circular holes were made with the awl in a cadaver. They were made in

PLATE 16. Awl used to demonstrate Langer's lines, and inch square ink stamp.



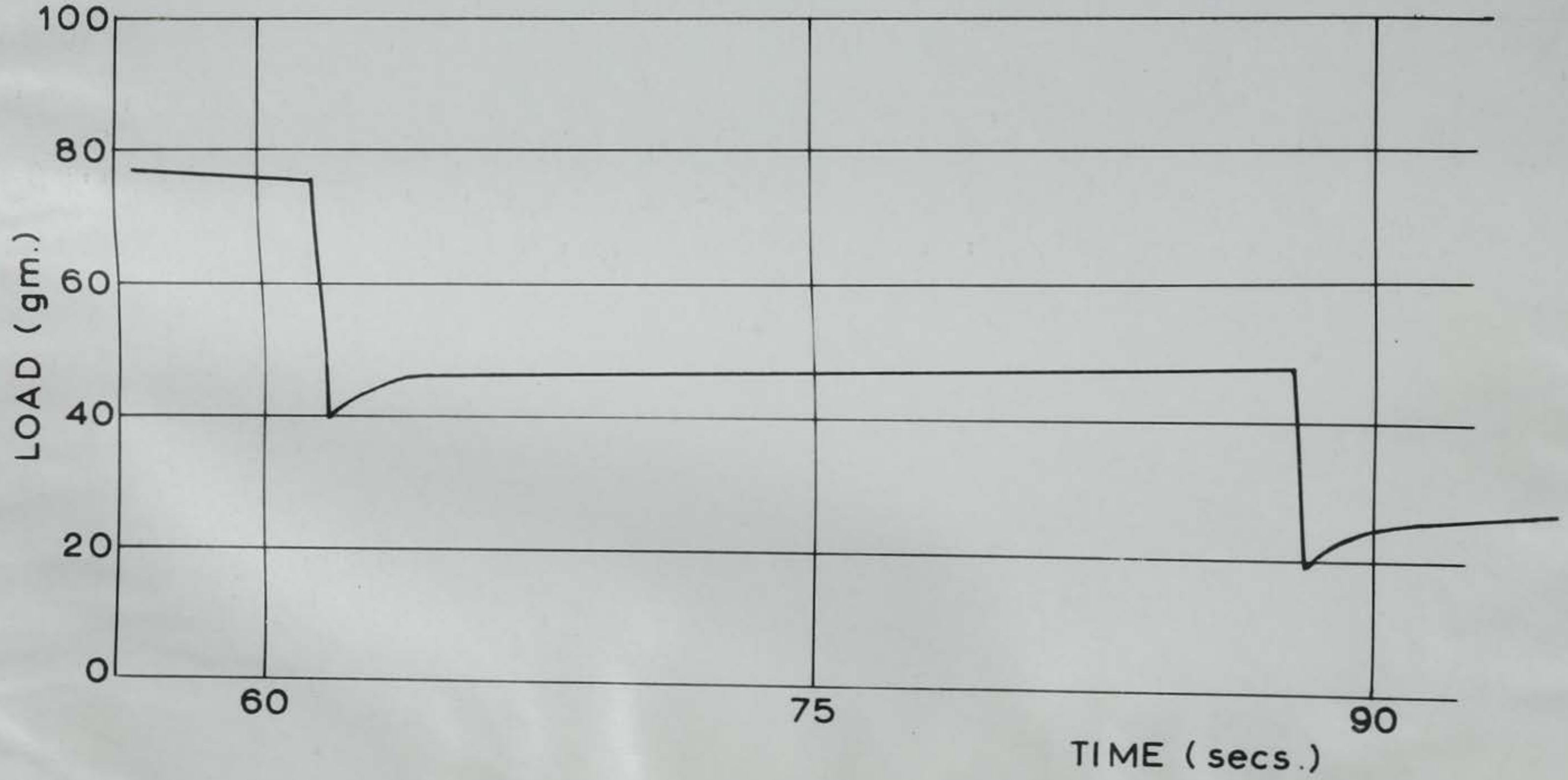


FIG. 31

STRESS RECOVERY

abdominal skin in the left hypochondrium and in the skin of the forearm. It can be seen that these holes assumed an eliptical shape. Plate 17 shows the effect of a number of these holes in the abdominal skin and plate 18 shows the effect of this more clearly in the skin of the forearm. Particular attention was paid to obtaining an accurate direction for the sites from which skin samples were taken. It has been shown that the direction of Langer's lines was well defined and constant for these particular sites.

An interesting modern parallel to the historic findings of Dupuytren (1834), who observed that the wound produced by a circular stiletto assumed a linear configuration suggesting it had been made with a bladed instrument, has been noted in an autopsy performed by Dr. P.N. Cowen in the mortuary of the General Infirmary at Leeds.

A patient was admitted to the hospital following a motor-car accident. The only injuries appeared to be a lacerated knee and a 2cm. cut on the chest (plate 19). The patient died within two hours. Enquiry revealed that the patient had been a passenger in the car, and that on impact, had been thrown forward on to the gear lever, which was of the column change type. The knob, which was circular had penetrated the chest, producing what was

PLATE 17. Eliptical shape assumed by circular puncture wounds in abdominal skin due to directional effect of Langer's lines.

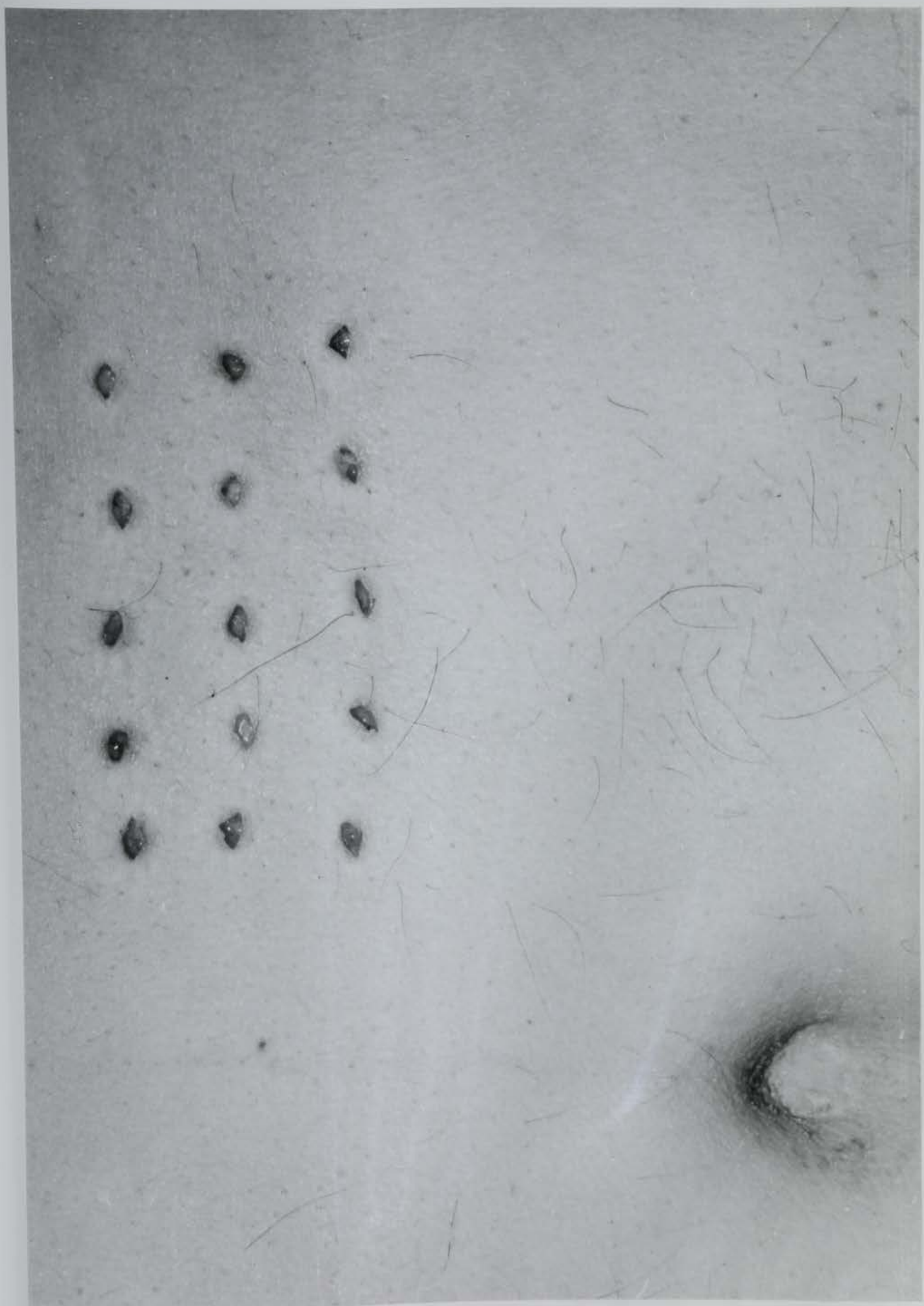
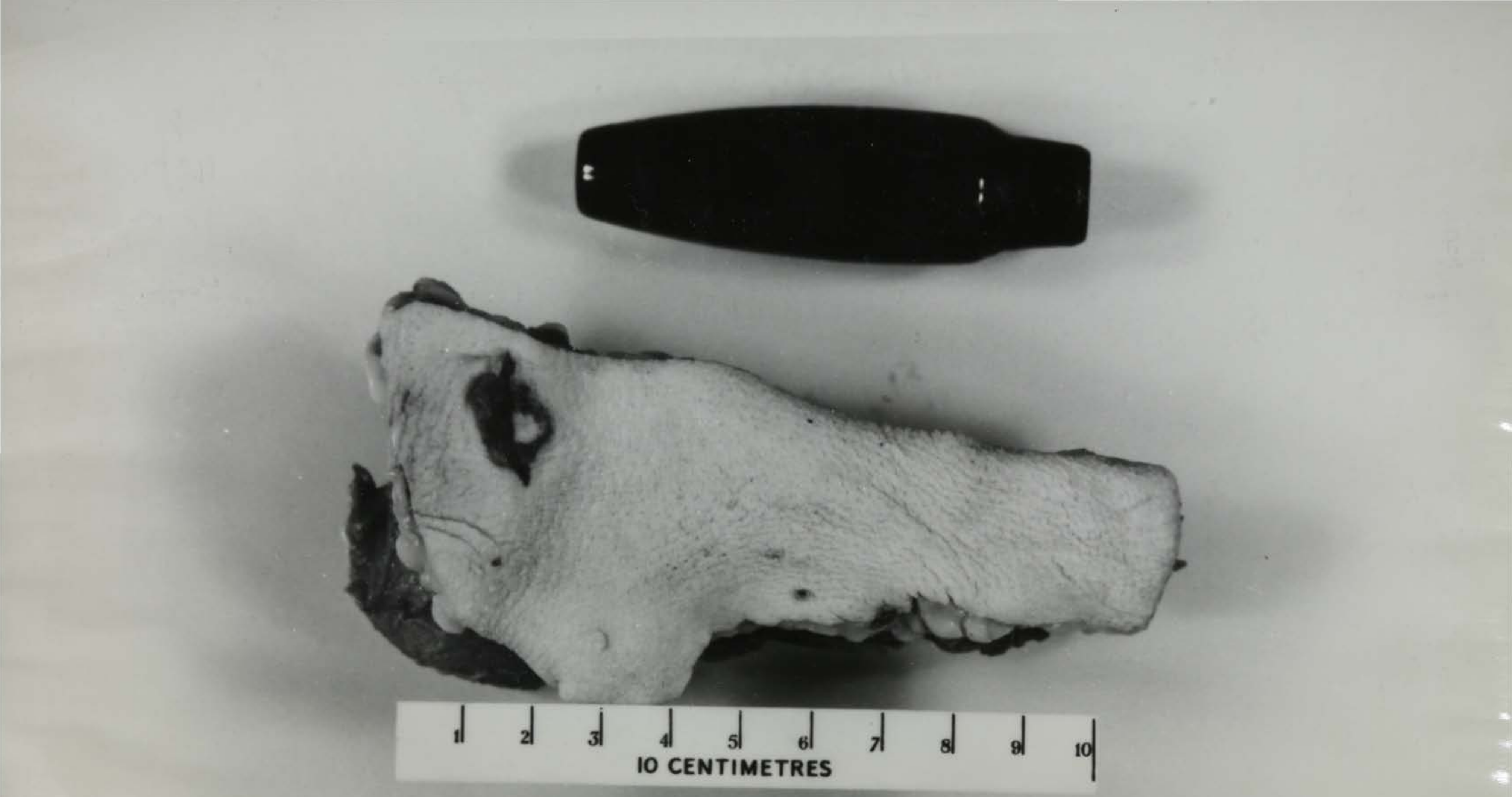


PLATE 18. A series of circular puncture wounds were made in the forearm. The linear configuration assumed is due to Langer's lines.

PLATE 19. Injury produced by rounded gear lever (shown on left). The injury was first attributed to a cut - the directional effect of Langer's lines.


1
2
3
4
5
6
7
8
9
10
10 CENTIMETRES

first thought to be a cut, rather than circular wound.

Tests were carried out to investigate the effect of the direction in which the specimens were cut relative to Langer's lines. A large piece of abdominal skin was obtained and numerous samples cut along and across the direction of Langer's lines. These were tested in the normal way on the extensometer. It was observed from the stress strain curves that the extension for a given load for specimens across Langer's lines was approximately twice that for specimens cut along the lines. The values of the constants were calculated (Table 2) and it was found that for the two directions the value of b was identical. However, the values of k and c differed.

	l	k	b
Female abdominal (88)			
along	0.451	0.43	0.16
across	0.44	0.64	0.16
Male abdominal (44)			
along	0.68	0.36	0.38
across	0.69	0.62	0.37
Female forearm (68)			
along	0.68	0.52	0.40
across	0.69	0.63	0.41
Male forearm (77)			
along	0.64	0.40	0.39
across	0.61	0.69	0.39

Table 2. Rheological constants along and across Langer's lines for the abdominal skin of two subjects and the forearm skin of two subjects. The age is shown in parenthesis.

This directional effect is clearly seen in figure 32 which shows results from the two directions plotted with the axes of log extension and log load.

It is seen that the two lines are parallel and consequently have the same gradient, indicating the same value of b. However the results from the specimens across Langer's lines produced the higher line, indicating a larger value of k.

10. Comparison of autopsy and biopsy specimens.

Of the specimens tested most were obtained at autopsy. This had the advantage that they could be taken from the exact position required. Some biopsy material was used which had been obtained from abdominal operations.

This had the disadvantage that the position and direction varied with the nature of the operation.

It was however shown that once compensation had been made for the direction in which the specimen was cut within a given area, similar results were obtained from both autopsy and biopsy material. Table 3 shows the comparison of results obtained from abdominal skin obtained from a 52 year old man undergoing a laparotomy with the mean results obtained from autopsy material for a similar site and age.

	l	k	b
biopsy	0.70	0.45	0.34
autopsy	0.68	0.43	0.35

Table 3. Rheological constants obtained from specimens of abdominal skin in two men age 52 years obtained at operation and autopsy respectively.

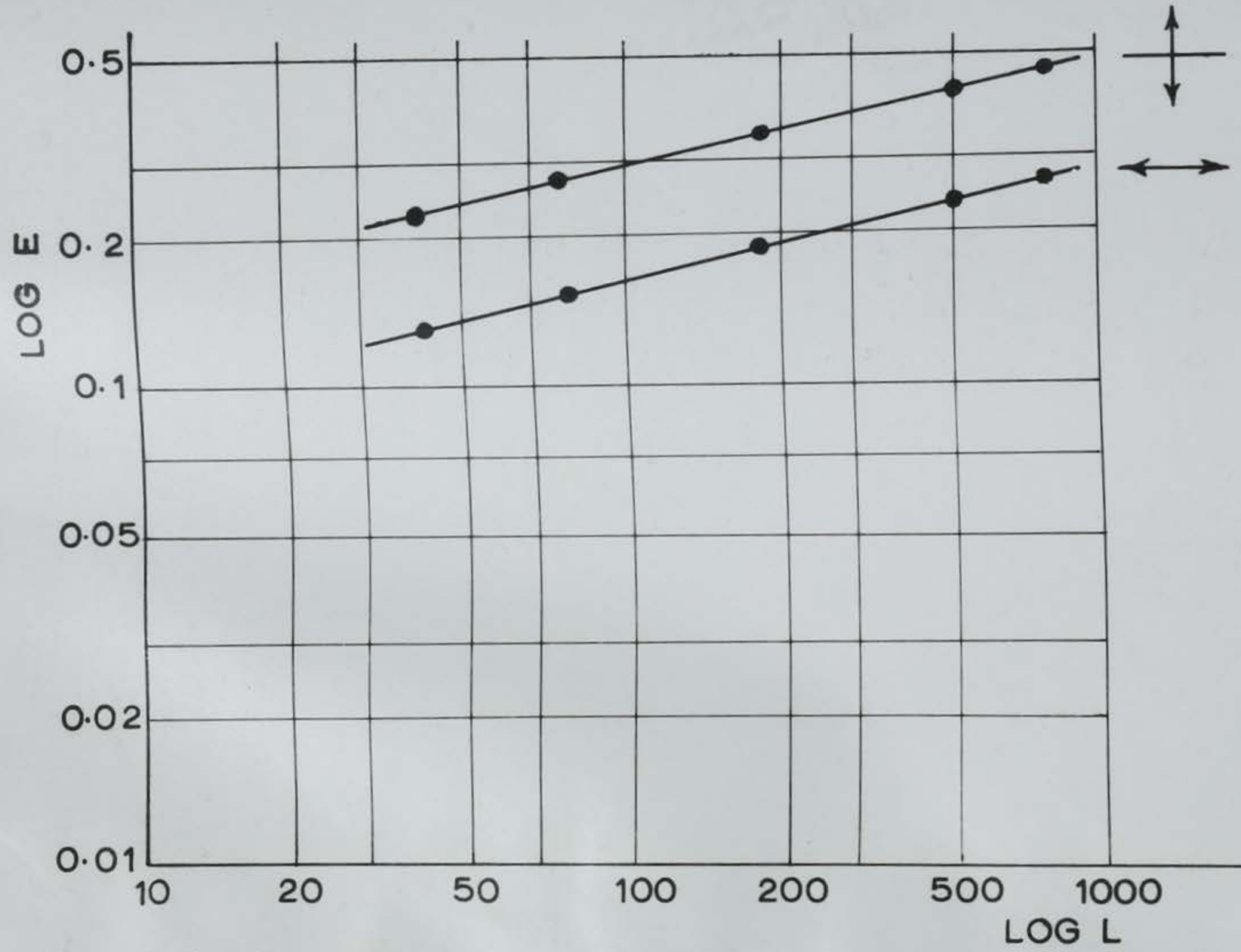


FIG. 32
LOG E LOG L ALONG (↔) AND ACROSS (↕) LANGER'S LINES
66 YEAR MALE ABDOMEN

11. Effect of freezing and length of storage.

Tests were carried out to investigate the effect of freezing on the properties of skin. A large piece of abdominal skin was taken at autopsy from a woman age 54 years. This was divided into twenty two specimens, half were cut along the direction of Langer's lines and half were cut across. Approximately half of each group was taken and tested while the other half were frozen in the normal way for twelve hours, and then tested. The values of the constants were not altered by freezing (Table 4).

In a further experiment fourteen specimens were prepared in the normal way from the abdominal skin of a 59 year old man.

	l	k	b
Frozen along	0.62	0.48	0.40
across	0.64	0.67	0.39
Unfrozen along	0.61	0.48	0.40
across	0.62	0.70	0.40

Table 4. The effect of freezing on the rheological constants along and across Langer's lines for the abdominal skin of a 54 year old woman.

Half of these were tested and the other half re-frozen for twelve hours and then tested. The mean results obtained after the first freezing were similar to those after the

second (Table 5).

	l	k	b
Frozen once	0.60	0.28	0.29
Frozen twice	0.63	0.29	0.28

Table 5. Effect of a second freezing on the rheological constants of abdominal skin from a 59 year old man.

At a storage temperature of -10° C. the skin began to deteriorate after a period of about 48 hours. This is demonstrated by the fact that after this period the skin was much more extensible and usually yielded prematurely. It was found however, that skin could be kept for much longer periods if stored at a lower temperature of about -50° C. Specimens yielded normal results after as long as 90 hours under these conditions. This was the longest period for which specimens were kept.

B. TESTS ON RAT TAIL TENDON

Rat tail tendons form a useful source of collagen fibres and this material was used to determine the mechanical properties of pure collagen fibres which are known to be orientated. This work is of value in investigating the problems of the structure of skin since once the properties of collagen are known its effects may be considered in relation to other components of skin.

For the tests tails were obtained from adult albino rats. The tails were stored under saline at room temperature until required for use, but were always tested within six hours of amputation. The tip of the tail was removed and collagen fibres obtained from the remaining portion with forceps. The fibres were usually withdrawn in bundles which could easily be split.

The fibres were mounted between the jaws of the extensometer with small pieces of chamois leather wrapped round their ends to prevent damage by the serrations of the jaws. The fibres were extended at a constant rate under saline and a typical stress strain curve is shown in figure 33. It was found that the power law of the form

$$E = kL^b$$

characterised the stress strain curve.

Tests were carried out to investigate the effect of

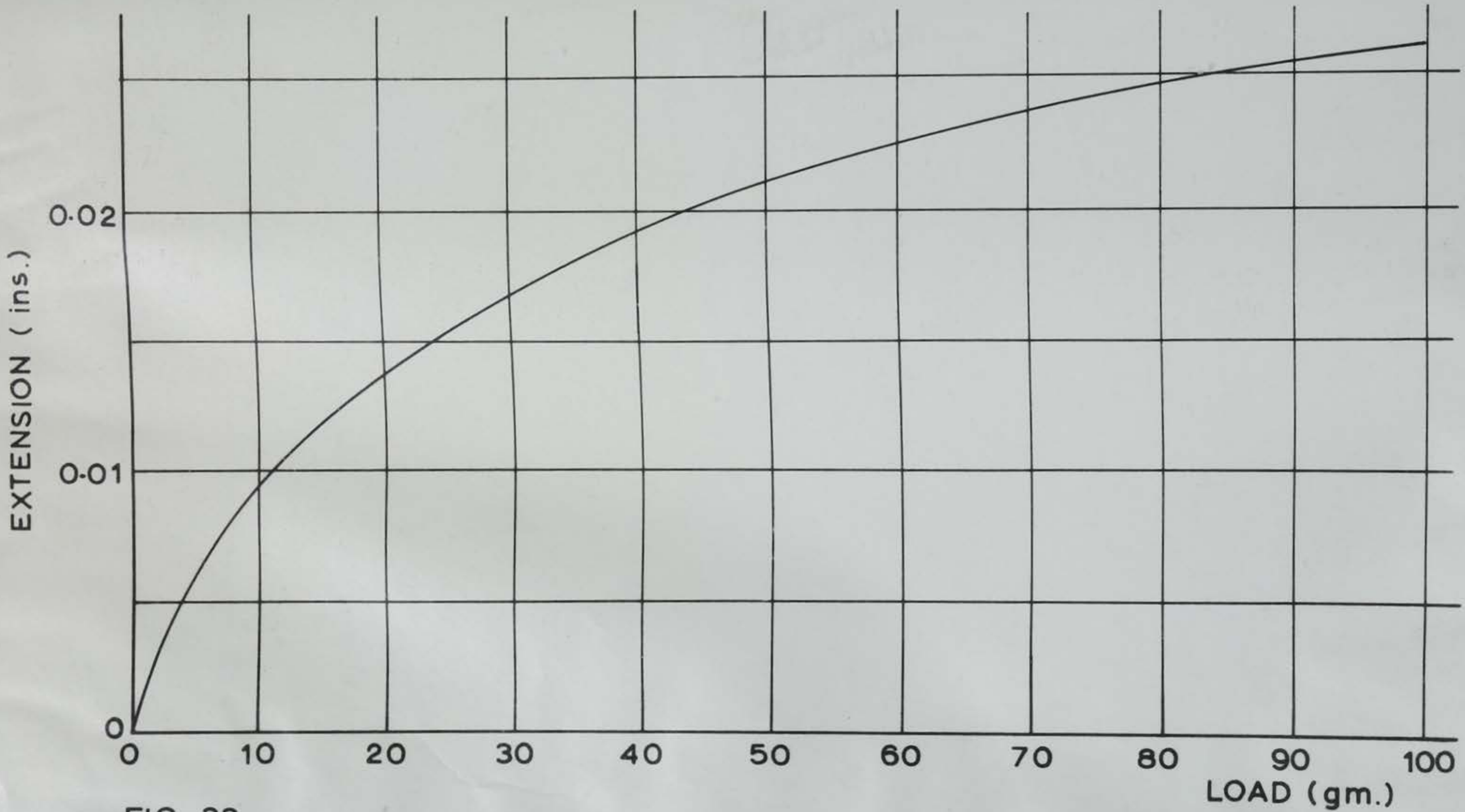


FIG. 33

LOAD/EXTENSION CURVE FOR RAT TAIL TENDON

freezing on the properties of rat tail tendon collagen fibres. A fibre was selected, mounted in the extensometer under saline, and extension applied at the rate of 8 inches per minute until a load of about 50gm. had been produced, this being approximately half the maximum load that the fibre was expected to carry. At this stage the extensometer was reversed, and run back to the initial position. The jaws were then removed from the extensometer with the fibre still mounted between them, placed in a beaker of saline and frozen at -10° C. for 24 hours. The jaws were then remounted in the extensometer and the test resumed to the point of rupture.

On plotting the results on log graph paper it was shown that the line produced from the second test became tangential with that of the first test (figure 34). As indicated in the section on progressive loading (V.A.4) this demonstrated that the rheological characteristics of the specimen were not affected by freezing.

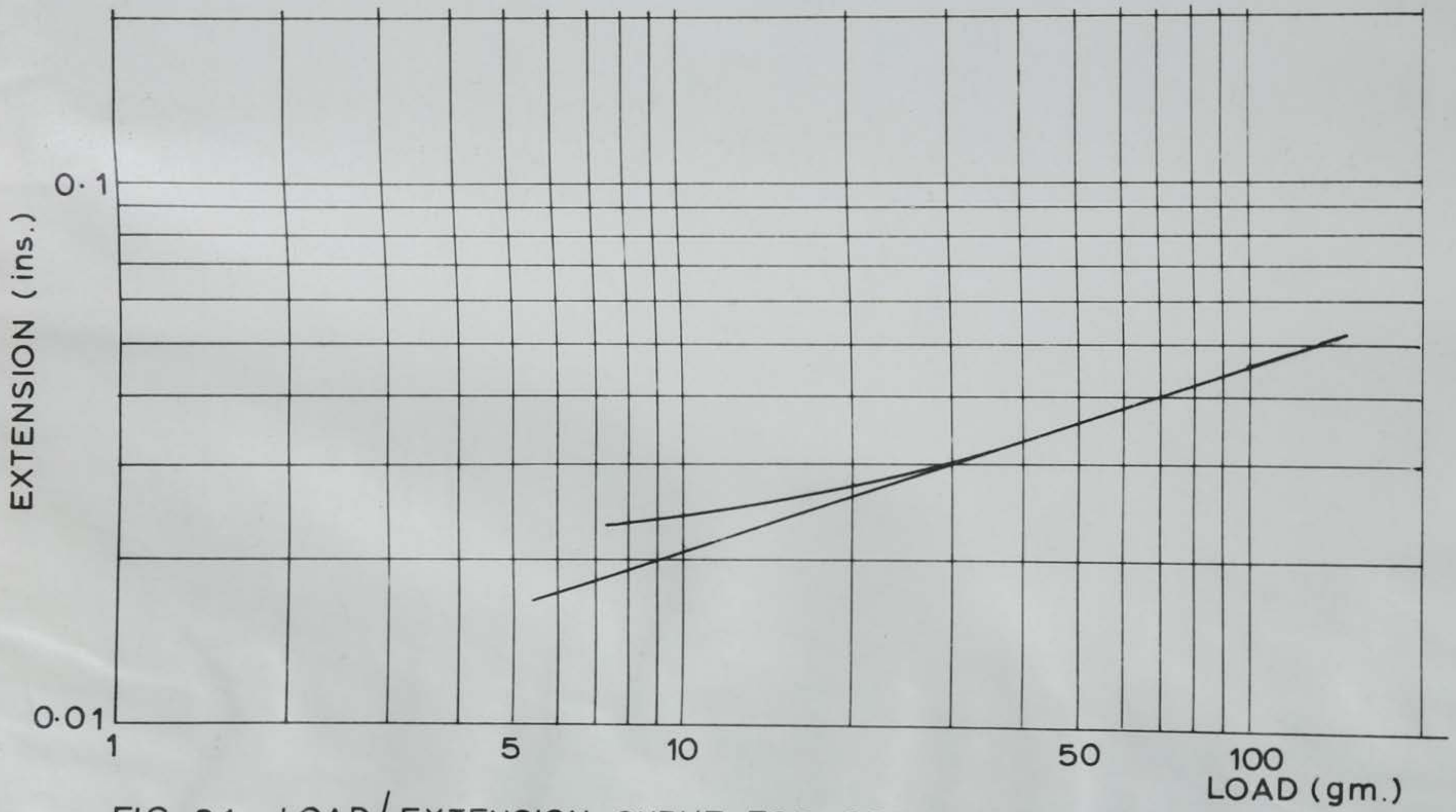


FIG. 34 LOAD/EXTENSION CURVE FOR COLLAGEN FIBRE

C. MECHANISM AND CHARACTERISATION OF EXTENSION OF SKIN.

Skin consists of a fibrillar meshwork of collagen and elastin surrounded by a gelatinous compound, consisting of polysaccharides, known as ground substance. The fibres may be seen microscopically to be of considerable length, frequently splitting or joining up into other fibres. This is similar to the structure of felt, discussed by Baines (1953).

Plate 20 shows a histological section taken from one of the specimens used in this study, stained by the Mallory method (appendix 2). This is the same technique as that used by Kenedi (1964b). It has been shown for skin (Rothman, 1953) that there is approximately twenty times more collagen than elastin by weight and its tensile strength is greater by a factor of about one hundred (Krafka, 1942). The effect of elastin on the more highly loaded part of the stress strain curve will therefore be negligible. In this part of the curve skin, for mathematical purposes, has been considered as a collagenous meshwork surrounded by viscous ground substance.

The extension of a piece of skin falls into two clearly defined modes:-

1. Aligning or orientation of the fibres
2. Extension of fibres

PLATE 20. Histological sections of skin stained with Mallory trichrome stain (magnification X850). The section on the left is across Langer's lines. The section on the right is along Langer's lines.

1. FIBRE ORIENTATION

In this instance the extension of a meshwork is considered, being analogous to the work carried out by Weisenberg (1949) and Shorter and Weisenberg (1949) who investigated the results of testing specimens of cloth cut at various angles with respect to the warp. In the case of skin it was assumed that the fibres do not extend during this stage but will behave as the laths in a garden trellis and will turn to become parallel with the direction of extension.

Histological examination of specimens before and after loading showed that the fibres do indeed become orientated in this way (plates 21 a and b).

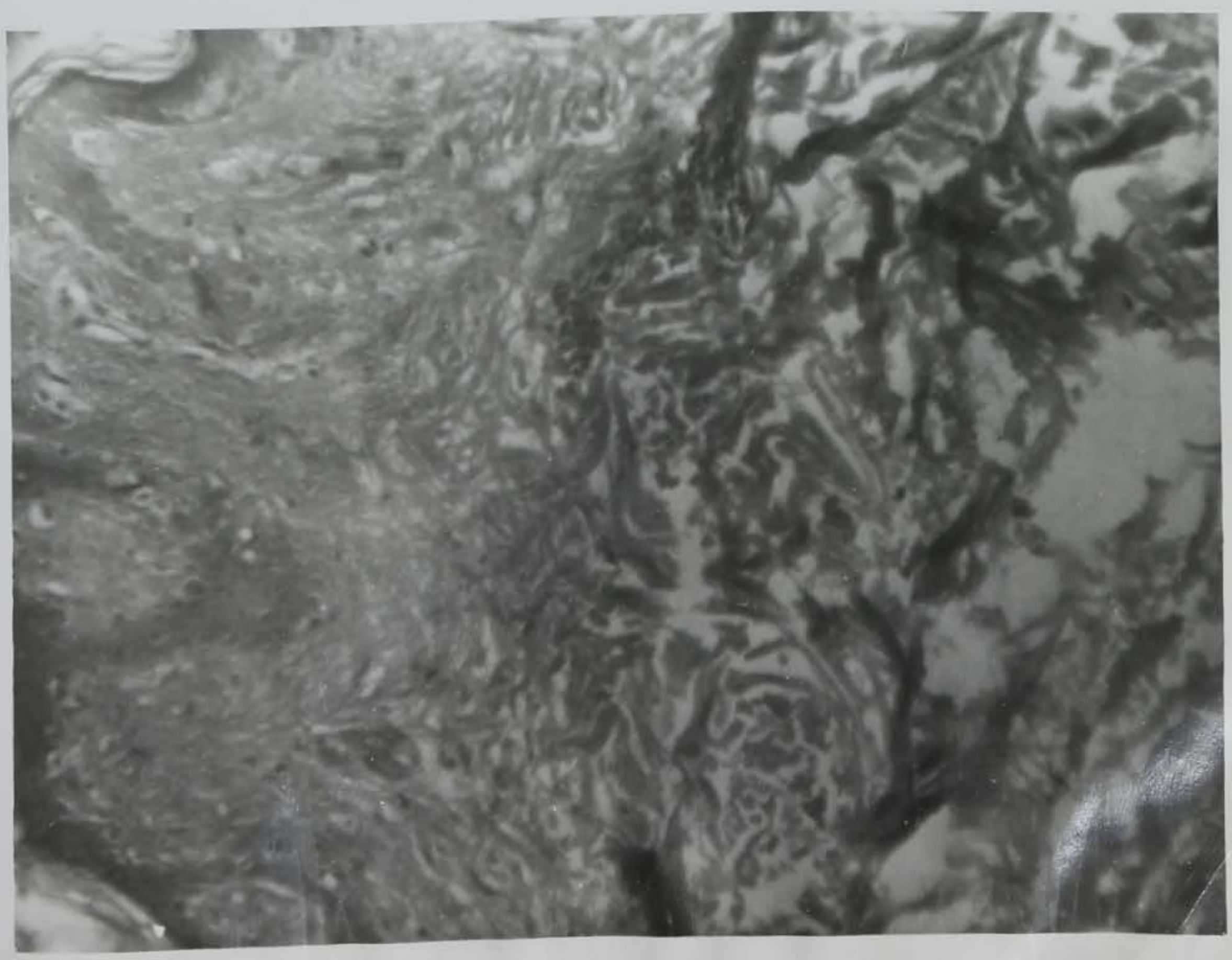
With specimens of skin of the type used in the present experiments these conditions prevailed up to a load of about 100 grams (figure 35) and the law

$$E = a * b \log L$$

where a and b = constants
E = extension
L = load

was found to apply well over all but the lowest portion of this range. The constants could be deduced by taking the gradient and intercept of the line produced by plotting the results on semi-logarithmic graph paper (figure 36).

PLATE 21. Histological sections of skin in the extended position a) along Langer's lines (upper section) b) across Langer's lines (lower section).



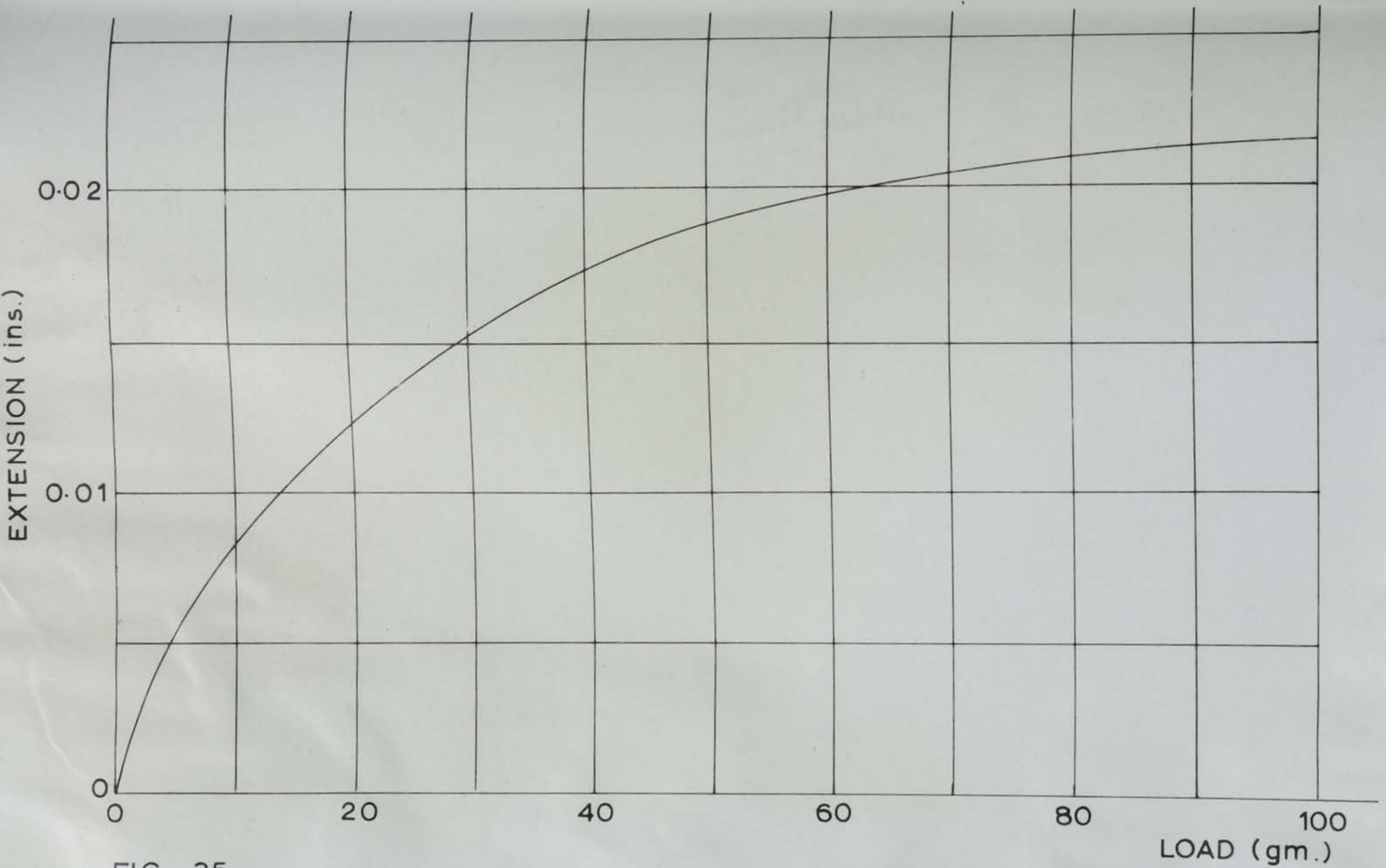


FIG. 35

INITIAL PORTION OF STRESS STRAIN CURVE FOR SKIN

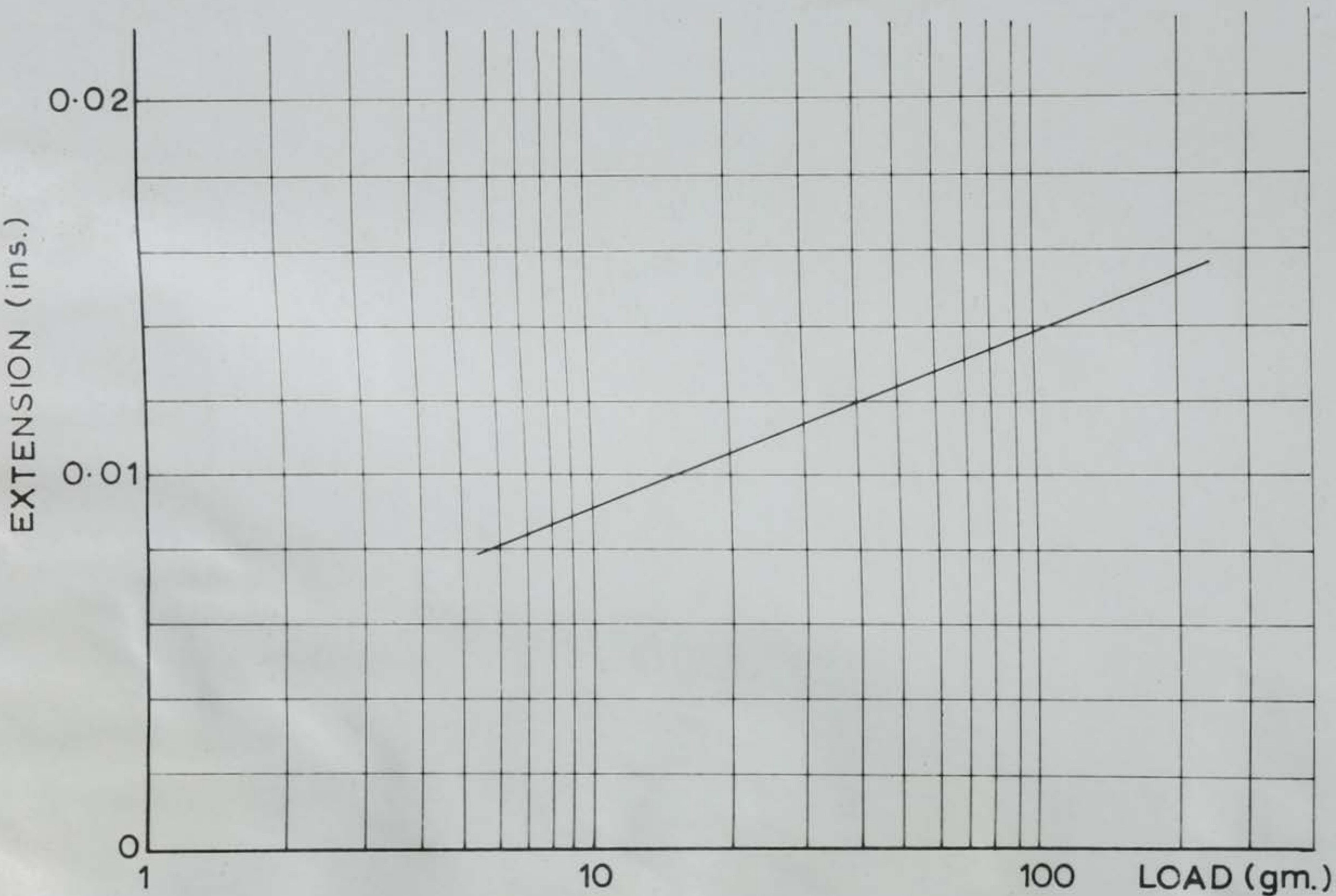


FIG. 36 GRAPH OF EXTENSION / LOG. LOAD

2. EXTENSION OF FIBRES

The second stage of extension commenced at the point where all the fibres had become orientated in the direction of the pull, but may still be considered to be unstretched. Thus the specimen at this point consisted of many parallel and straightened fibres which were in the unloaded stage. Within the load range from approximately 100 grams to a point just prior to the commencement of yielding (usually in excess of 1000 grams) it obeyed the law

$$E = k L^b$$

where E = extension
L = load
k & b = constants

This equation is verified by plotting the results from the stress strain curve on double logarithmic graph paper, where k is represented by the intercept and b the gradient (figure 37).

Since the specimens at this stage were effectively orientated collagen fibres, this prompted the investigation of the characteristics of pure collagen procured from a different source. The studies of collagen from the tendons of rat tails have been described in the previous section. The stress strain curve was found to be characterised by a power function of the same form as for skin in the second stage of extension.

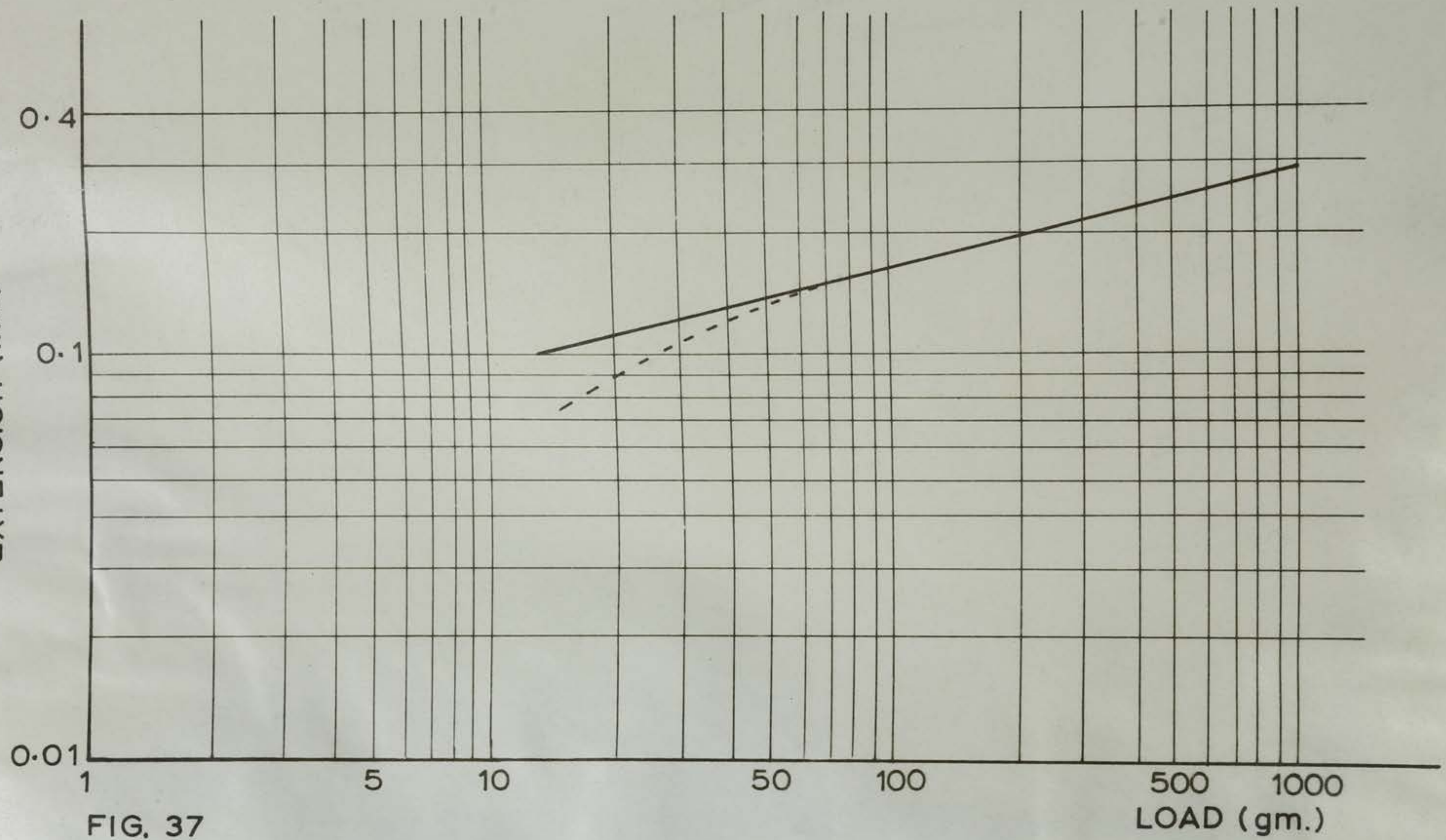


FIG. 37

LOG E/LOG L 66 YEAR MALE ABDOMEN

3. INFLUENCE OF DIMENSIONS OF SPECIMEN.

Area.

This area is the cross-sectional area of the specimen which when divided into the load gave the stress,

$$\text{i.e. } \frac{\text{load}}{\text{area}} = \text{stress (gm / sq.cm.)}$$

We will consider some ideal specimen of unit area and standard length which obeys the law:-

$$E = L^b$$

throughout its complete range (figure 38). If now the area of such a specimen is changed by a factor 'n' a load of nL will be required to produce the extension which could previously have been achieved with a load of L. This may be considered analogous to having n specimens of unit area in parallel. Thus the equation of the curve produced by such a system is

$$E = \frac{L}{n}^b$$

i.e. if the area is altered by a factor n the extension is modified by a factor $\frac{1}{n}^b$ for any given load.

If the result is now plotted next to the original one on log paper it is seen that there are two parallel lines, of gradient b, but that the second is shifted horizontally from the first by a factor n (figure 39).

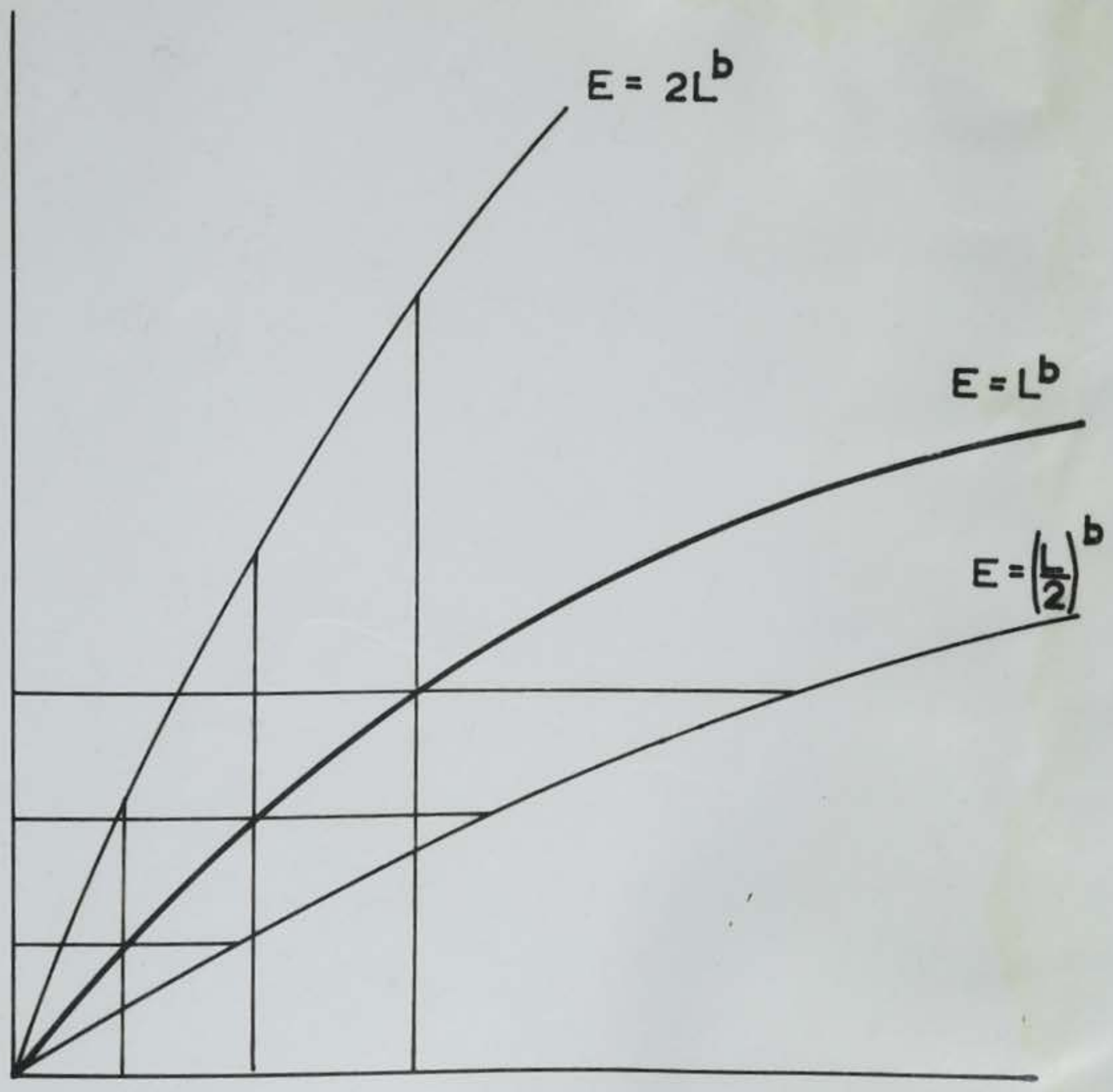


FIG. 38

$$E = \frac{a}{n^b} L^b$$

LET $\frac{a}{n^b} = k$

$\therefore E = kL^b$

a = FIBRE LENGTH

n = TOTAL AREA OF FIBRES

DIMENSIONAL EFFECTS OF SPECIMEN

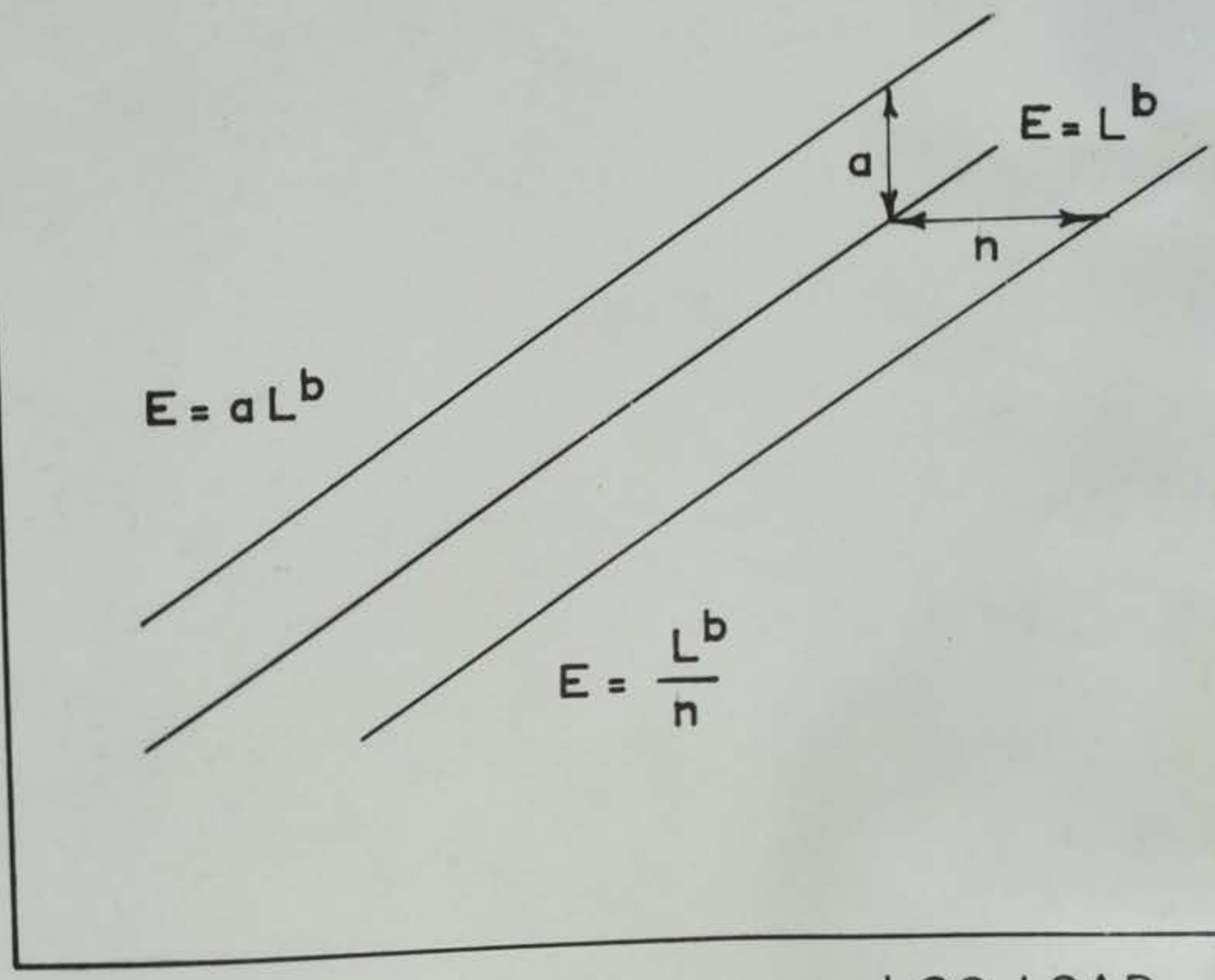


FIG. 39

SHIFT DUE TO DIMENSIONAL FACTORS

Length.

If the ideal specimen is considered again, it is seen that if the length of the specimen is altered by a factor 'a' the extension will also be altered by this factor. This may be compared with a system having 'a' specimens in series subjected to a load L. The resulting equation will be of the form

$$E = a\,(L)^b$$

If this result is plotted on the log paper and compared with the original equation, it is seen that again two parallel lines of gradient b are obtained, but that in this case a vertical shift of a is obtained (figure 39).

Combining the two results for length and area, the original equation becomes modified to

$$E = a\,\frac{L}{n}^b$$

Application.

1. The index b is independent of the dimensions of the specimen, thus if we are only interested in values of b dimensional inaccuracies are unimportant.

2. If we require a more fundamental analysis, we may compensate for known variations of specimen size.

3. If specimens of identical size are used n will give information about the relative area of collagen fibres under stress and, in the case of two specimens cut at 90°

to each other from the same piece of skin, n will give an indication of the degree of orientation with regard to Langer's lines.

4. EVALUATION OF CONSTANTS

In this analysis the following notation will be used.

D = fibre density normal to the direction of orientation

l = length of specimen as cut

W = width of specimen as cut

ϕ = the mean orientation angle - angle between mean direction of fibres and Langer's lines

$a_{\parallel}$ = length of orientated unstretched fibre parallel to Langer's lines

$a_{\perp}$ = length of orientated unstretched fibre normal to Langer's lines

$n_{\parallel}$ = number of fibres in specimen parallel to direction of Langer's lines

$n_{\perp}$ = number of fibres in specimen normal to direction of Langer's lines

Figure 40a shows the assumed fibre pattern for a specimen which is cut normal to the direction of Langer's lines. It can be seen that this is in the form of a trellis which is to be pulled in the direction of the arrows.

If we consider one parallelogram (figure 40b) of semi-angle ϕ and width X in the direction of pull it can be seen that this will extend to $\frac{X}{\sin \phi}$ when all the links are

parallel to each other in the direction of pull. Similarly if n such parallelograms are put in series the total length will be nX and they will extend to $\frac{nX}{\sin \phi}$.

Returning to the specimen let nX = ℓ and we see that the specimen extends to $\frac{\ell}{\sin \phi}$

$$a = \frac{\ell}{\sin \phi} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

The number of fibres in the specimen which may be assumed to become parallel to each other and therefore are involved in carrying load during extension is equivalent to the number of fibres terminating at the end of a specimen of given width W (figure 41a)

$$n = WD \sin \phi \ldots\ldots\ldots$$

There exists however a second set of fibres running across the specimen as in figure 41b and therefore the total number of ends involved will be

$$n = 2\ WD \sin \phi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

For a specimen cut and extended in the direction parallel to Langer's lines similar reasoning to the above will produce the results

$$a = \frac{\ell}{\cos \phi} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

$$n = 2\ WD \cos \phi \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

combining (1) and (3)

$$\frac{a_{\parallel}}{a_{\perp}} = \frac{\ell}{\cos \phi} \times \frac{\sin \phi}{\ell}$$

$$\frac{a_{\parallel}}{a_{\perp}} = \tan \phi' \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

combining (2) and (4)

$$\frac{n_{\parallel}}{n_{\perp}} = \frac{2\,WD \cos \phi}{2\,WD \sin \phi}$$

$$\frac{n_{\parallel}}{n_{\perp}} = \frac{\ell}{\tan \phi} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

from (5) and (6)

$$\frac{a_{\parallel}}{a_{\perp}} = \frac{n_{\perp}}{n_{\parallel}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

now let

$$k = \frac{a}{n^b}$$

$$k_{\parallel} = \frac{a_{\parallel}}{n_{\parallel}^b} \text{ and } k_{\perp} = \frac{a_{\perp}}{n_{\perp}^b}$$

$$\frac{k_{\perp}}{k_{\parallel}} = \frac{a_{\parallel}}{n_{\perp}^b} \times \frac{n_{\parallel}^b}{a_{\parallel}} = \frac{a_{\perp}}{a_{\parallel}} \times \left(\frac{n_{\parallel}}{n_{\perp}}\right)^b$$

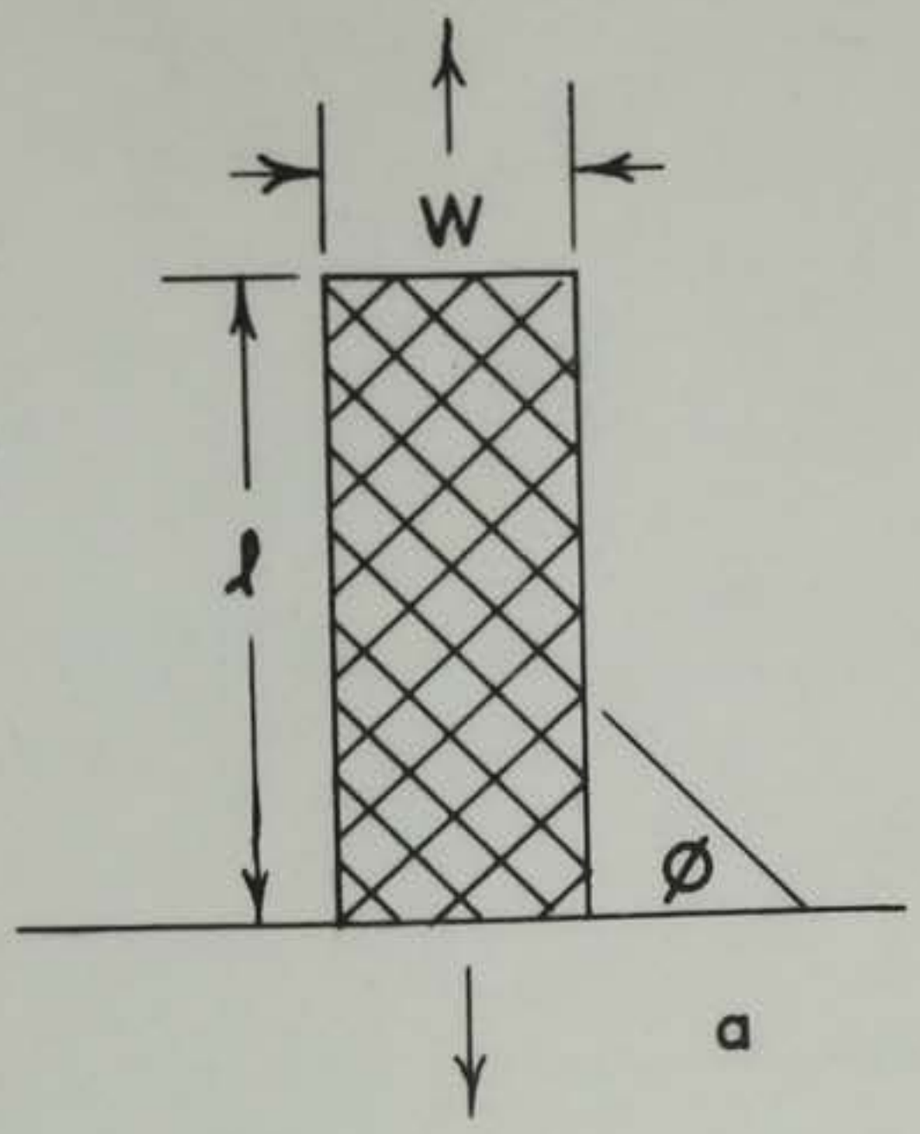
using equation (7)

$$\frac{k_{\perp}}{k_{\parallel}} = \frac{n_{\parallel}}{n_{\perp}} \times \left(\frac{n_{\parallel}}{n_{\perp}}\right)^b = \left(\frac{n_{\parallel}}{n_{\perp}}\right)^{b+1}$$

therefore from (6)

$$\frac{k_{\perp}}{k_{\parallel}} = \left(\frac{1}{\tan \phi}\right)^{b+1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Now $k_{\parallel}$ $k_{\perp}$ and b will be known from the solution of the results of two specimens cut from the same site but with



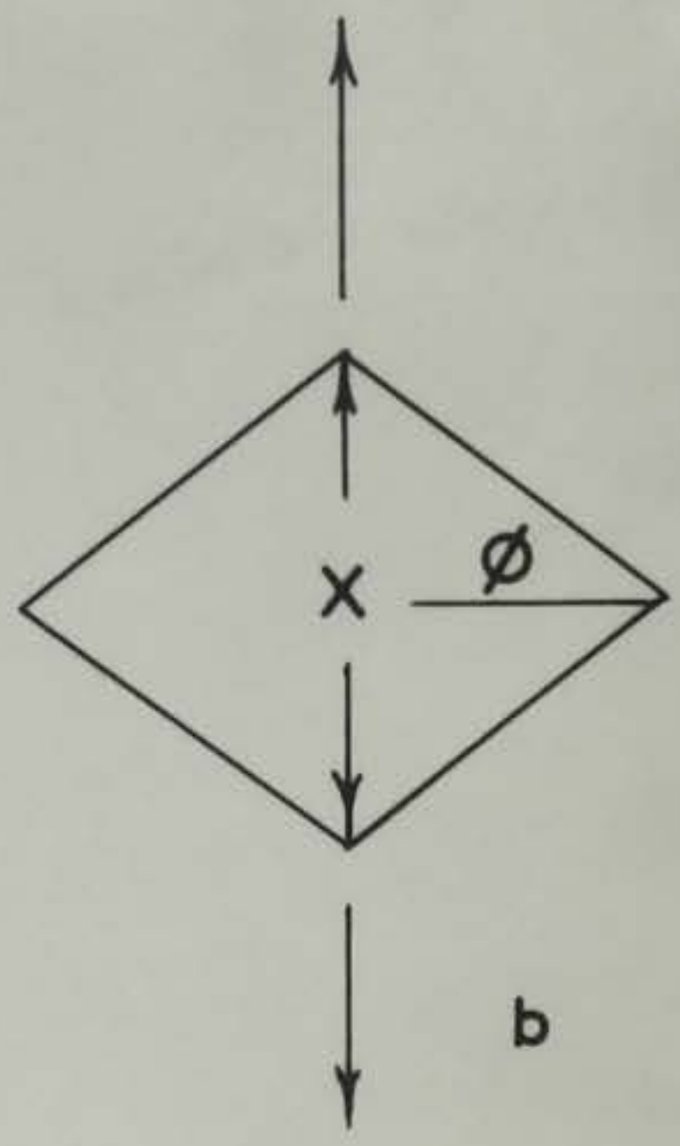
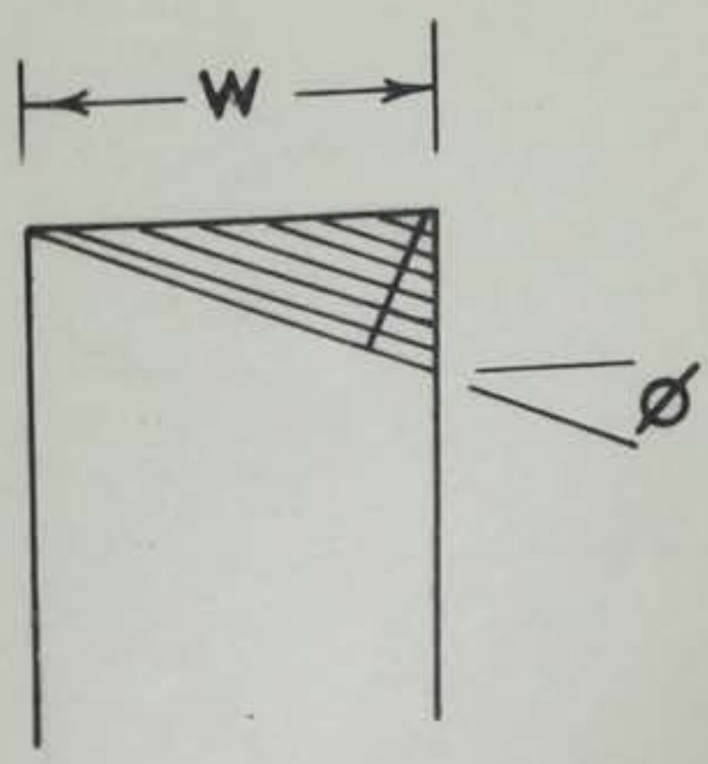


FIG. 40



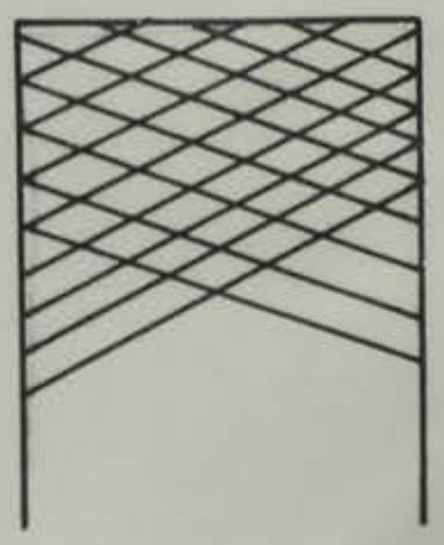


FIG. 41

MECHANISM OF EXTENSION OF SKIN

their directions along and across Langer's lines. Thus equation (8) may now be applied and the value of ϕ (mean orientation angle) may be found. Thence by the application of equations (5) and (6) the ratios $\frac{n_{\parallel}}{n_{\perp}}$ and $\frac{a_{\parallel}}{a_{\perp}}$ can be deduced. Alternatively, application of equations (1) and (3) will give absolute values of $a_{\parallel}$ & $a_{\perp}$. As the curve starts tangential to the "y axis" it is difficult to decide the exact point at which the specimen comes under load; a considerable initial extension produces an immeasurably small increase in load. Even such devises as increasing the sensitivity of the recorder by a factor of 100 failed to define accurately the starting point. The result of this is that we cannot measure the length of the specimen at zero load, the effect being to produce a curve with a false load axis.

In figure 42 the curve is drawn with reference to the L axis. However, the curve should have the axis L_2 as its datum, corresponding to zero extension at zero load, and all extension should be measured from this axis. Thus if the axes differ by an extension of c the true equation will be

$$E=c+a\left(\frac{L}{n}\right)^{b}$$

It is now necessary to be able to fit the above equation to an extension curve and to calculate the values

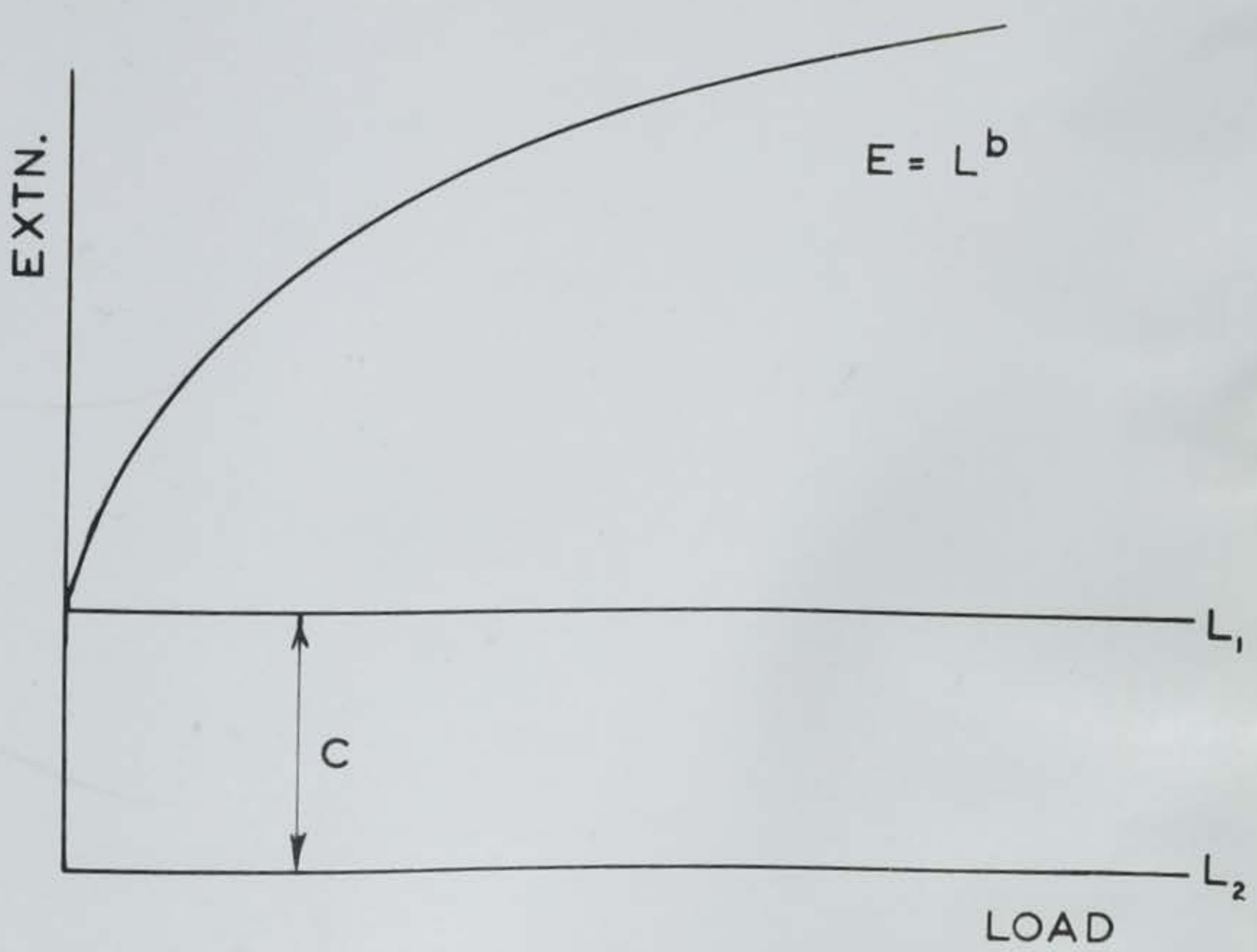


FIG. 42

ZERO ERROR

of the constants. As the equation stands the values of a and n cannot be derived because they form a quotient and therefore cannot be separated at this stage. The term $\frac{a}{n^b}$ will thus be regarded as a single constant denoted by k and the equation now becomes

$$E = c + kL^b$$

Various methods were used to obtain the constants, the problem being complicated by the fact that b represented a power which was considerably less than unity. The standard method of plotting on double log paper could not be used, since the value of c is not known and thus the ordinates are not completely defined. The methods finally evolved to obtain these constants are discussed in section V.D.

5. EFFECT OF SKIN TENSION IN THE BODY.

After the removal of skin at autopsy or biopsy it was evident that shrinkage occurred due to the relaxation of natural tensions within the skin. To demonstrate this an instrument consisting of two scalpel blades held exactly one inch apart was constructed. With this two incisions were made simultaneously in the skin of the fore-arm and abdomen of cadavers in a given direction and the resulting shrinkage of the strip of skin measured while it was still in position under the action of the normal tension, along its length. A further two incisions were then made at right angles to the original ones to remove all the tensions from a square of skin. It was found that as the incisions progressed across the width of the original strip a stress concentration was formed in the uncut portion of the stress piece, resulting in a trapezoidal form rather than a square. This was unsatisfactory for the purposes of measurement and an alternative method was used.

An ink stamp was constructed which marked a one inch square on the surface of the skin with a line across its centre to indicate the direction of Langer's lines (plate 16). It was found that the surface of the skin could be marked in this way with virtually no deformation (plates

22a and b). When the lines of the square were carefully excised with a sharp scalpel the resulting test piece represented what had originally been one square inch, irrespective of the asymmetric tensions produced while cutting (plates 23a and b). The test piece was then removed from the cadaver and stripped of its subcutaneous fat. Measurements in the two directions along and across Langer's lines, were carried out with reference to the indicator mark across the centre of the specimen.

The results of such tests on squares of skin from the arms and abdomens of cadavers at various ages are shown in Table 6a.

Age (years)	40-49	50-59	60-69	70-79	80-89
MALE (forearm)					
along	0.82	0.87	0.70	0.90	0.89
across	0.90	1.0	1.1	1.0	1.0
MALE (abdomen)					
along	-	0.88	0.85	0.95	0.90
across	-	0.90	1.0	0.93	1.0
FEMALE (forearm)					
along	0.78	0.80	0.78	0.87	0.88
across	0.96	0.95	0.92	0.99	0.91
FEMALE (abdomen)					
along	0.85	0.85	0.85	0.73	0.88
across	0.91	0.93	0.90	0.95	0.88

Table 6a. The mean length to which one inch square specimens of skin shrank in the two directions (along and across Langer's lines).

PLATE 22a. Inch square stamp on forearm.

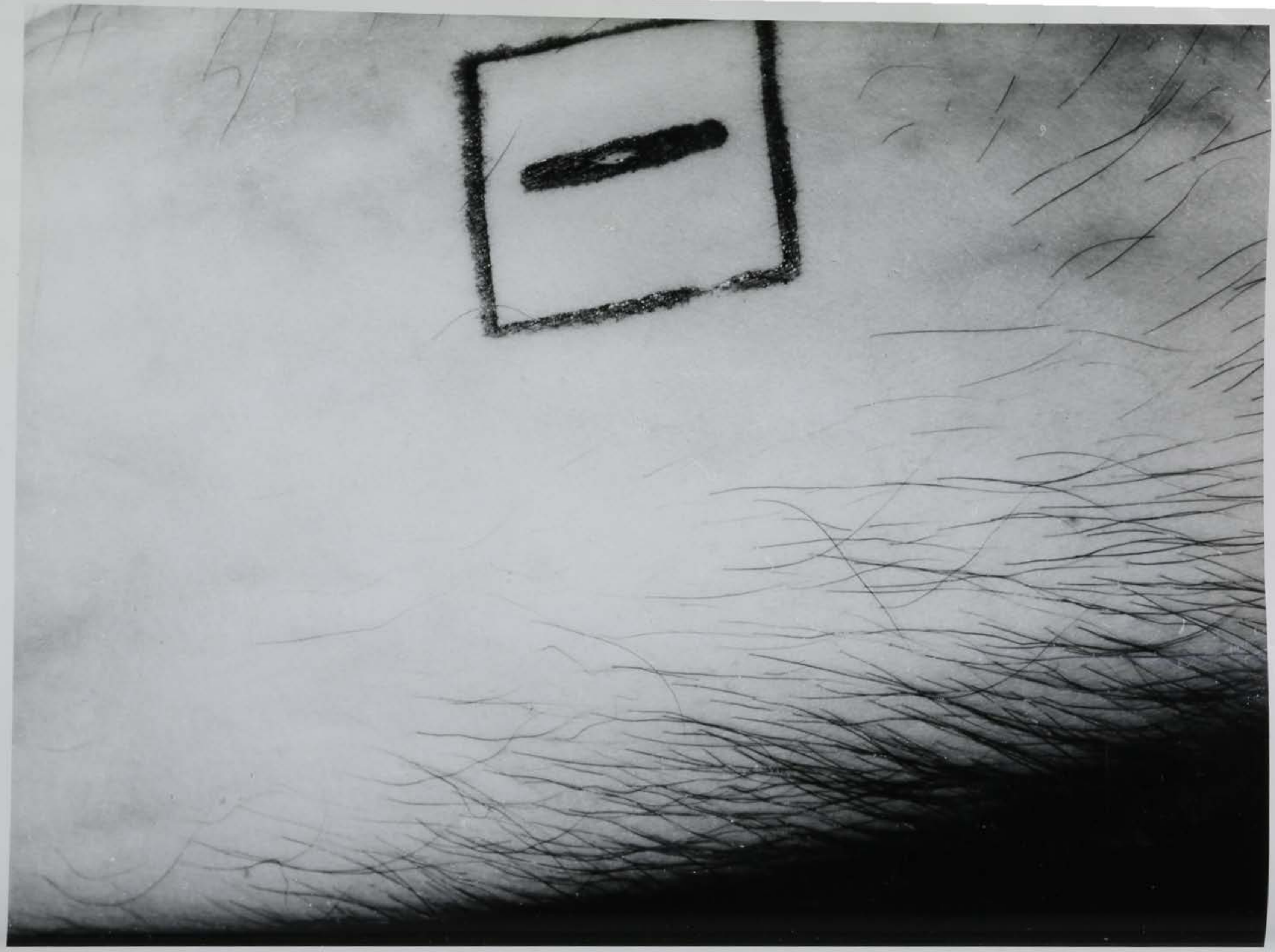


PLATE 22b. Inch square marked on abdominal skin.

PLATE 23a. Inch square on forearm incised along its borders. Note the retraction is greater in the direction of Langer's lines.

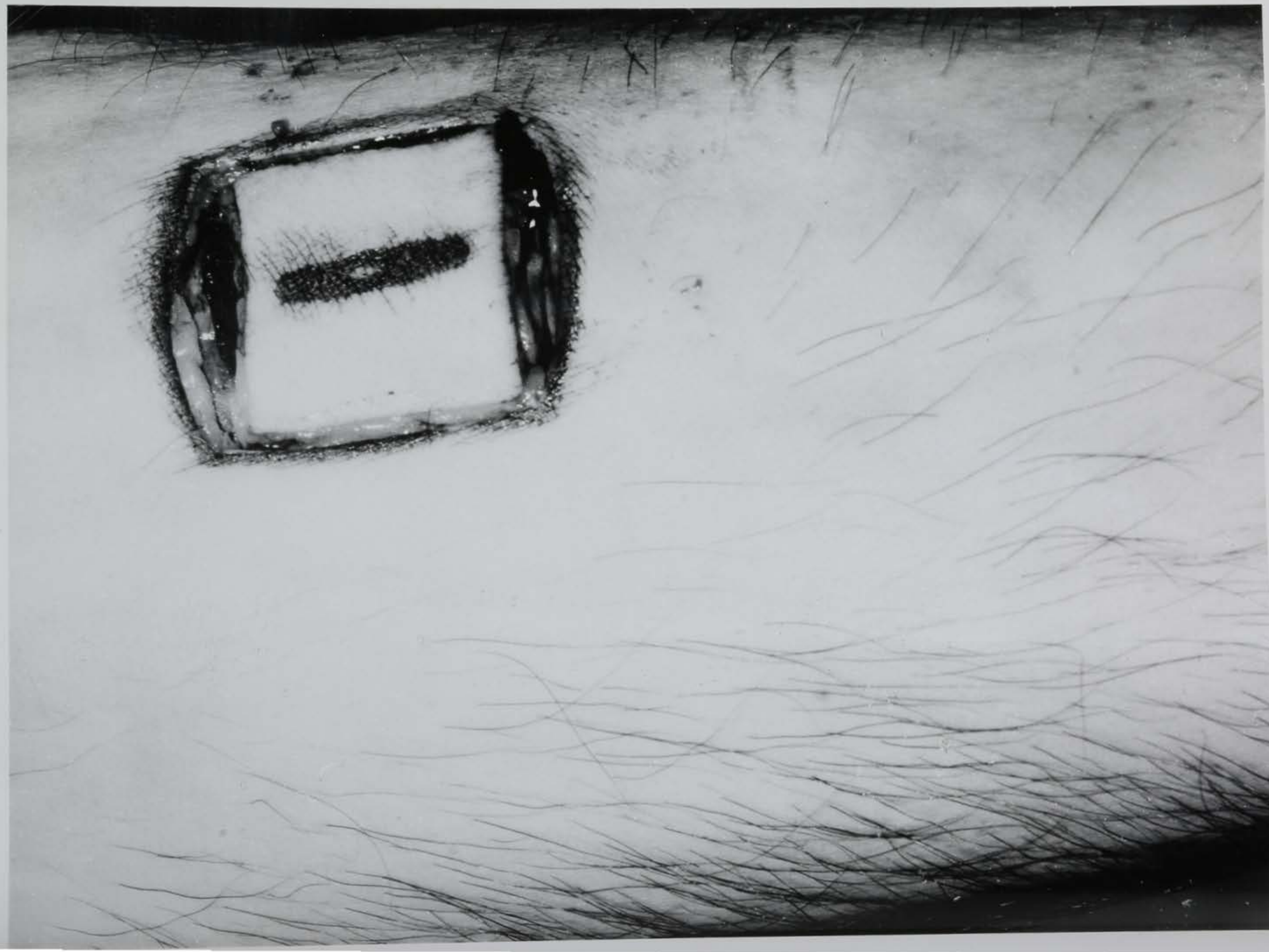
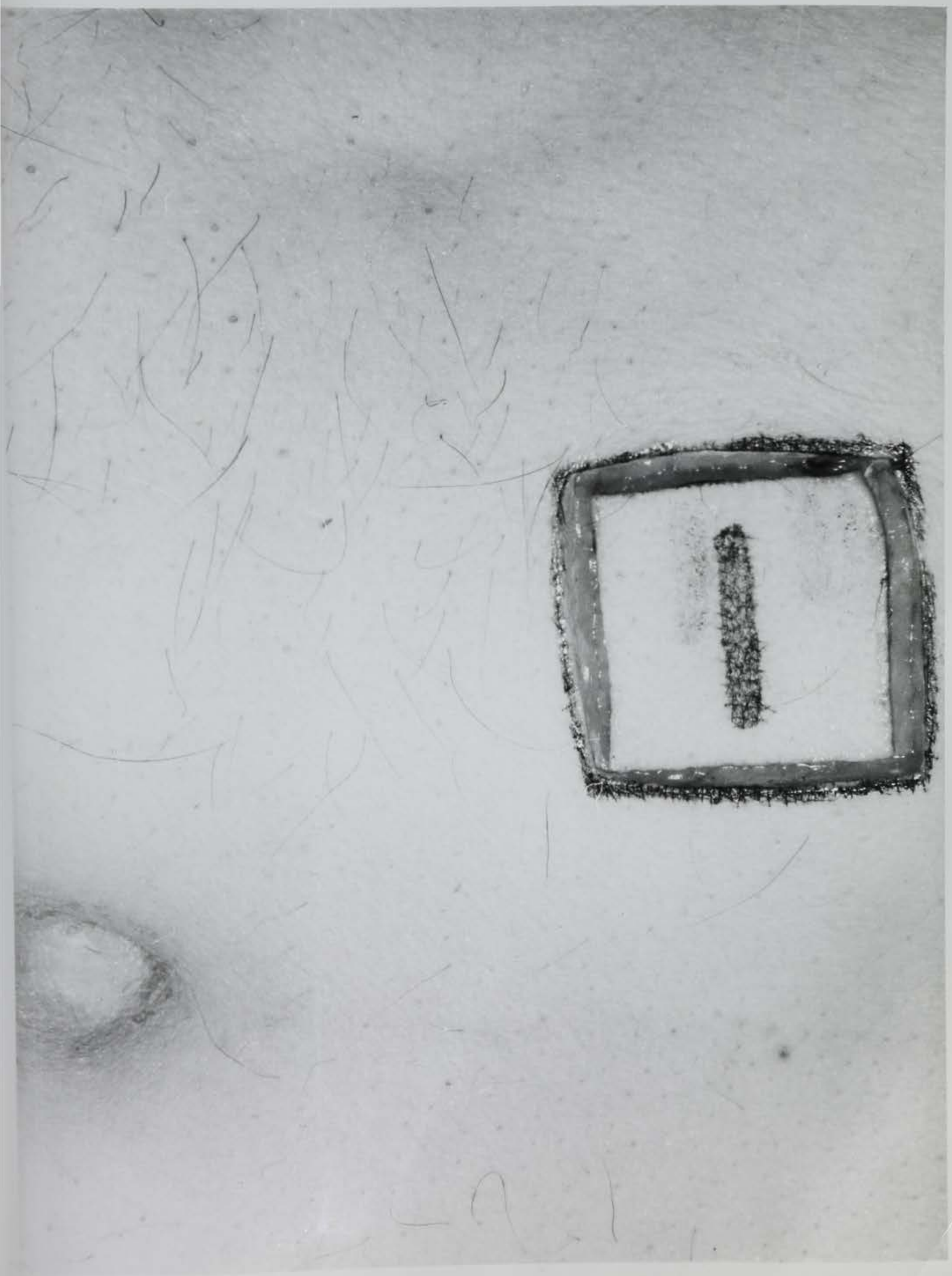


PLATE 23b. Inch square on abdomen incised along its borders. Note the definite retraction, although the directionsl effect of Langer's lines is not marked.



It was found that shrinkage in the direction of Langer's lines was greater than that at right angles to it. From the fifth to the ninth decades there was no obvious correlation with age. It was found that the obesity of the patient often appeared to be an important factor in the amount of tension. For instance in the forearm the mean retraction along Langer's lines was 0.24" in obese men compared with 0.13" in thin men. Similarly the mean retraction in the abdominal skin along Langer's lines in obese men was 0.25" compared with nil in thin men.

The skin from both the forearm and abdomen of men retracted more than that from women (Table 6b). The data were insufficient to determine whether this was an effect of obesity.

	Male	Female
Forearm		
along	0.07	0.14
across	0	0.09
Abdomen		
along	0.09	0.19
across	0.05	0.11

Table 6b. The mean retraction of the inch square of skin from the forearm and abdomen of men and women between the ages of 40 and 90 years measured along and across Langer's lines.

The pieces of skin used for ascertaining rheological characteristics had been subject to this type of shrinkage, since they were specimens excised from the body. The compensation which needs to be made to the constants l and n due to this dermal shrinkage is considered below.

Effect on the constant l.

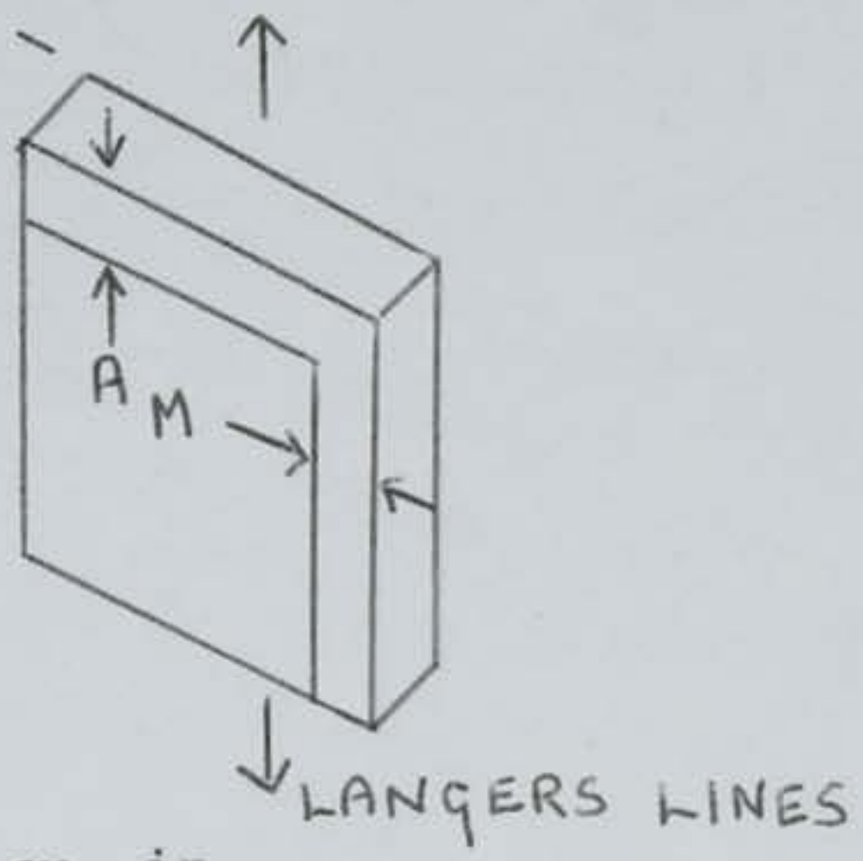
Consider two specimens which have been cut with the same die, one before and the other after shrinkage. The one which has been cut before shrinkage will shrink after cutting, but is extended to its in situ state when mounted on the extensometer, and the value of the constants l and n may be calculated. The specimen which has been cut after shrinkage will have a greater free length than the one which has shrunk after cutting, and will consequently give a higher value for l. It can thus been seen that the increase in l is directly proportional to the amount of shrinkage of the specimen.

Effects on the constant n.

Considering again the two specimens which have been cut before and after shrinkage, it is seen that the one that has been cut after shrinkage has been taken from a piece of skin which has changed in width and thickness and consequently the fibre density normal to the direction of the specimen has been changed. This will represent a reduction in the value of n as explained below.

Change in n due to dermal shrinkage.

Consider a square inch of skin of thickness t (diagram below)



Volume = 1.1t = t cu. in

now let the shrinkage in the direction of Langer's lines be A, that at right angles to them be M, and the new thickness be X

The new volume = (1-A)(1-M)X.

Assuming the volume does not change, then the above expansion will be equal to t

$$X = \frac{t}{AM-A-M+1}$$

The new area of the specimen normal to the direction of Langer's lines is then

$$X(1-A)$$

The original area was t

fibre density is reduced on removing the specimen from the body by a factor of $\frac{t}{X(1-A)}$

D. CURVE FITTING

Various methods of obtaining the values for the constants in the stress strain relationship have been evolved. These are outlined below.

1. Differential method.

If the equation is differentiated with respect to L an expression is obtained which is independent of c.

$$E = c + k L^b$$

$$\frac{dE}{dL} = b k L^{b-1}$$

The values of $\frac{dE}{dL}$ can be found by measuring the gradient of the stress strain curves at various loads. The graph of $\frac{dE}{dL}/L$ may now be drawn on double log paper (figure 43). The result is a line of gradient b - 1. Hence b and the intercept bk can be found, and the value of k calculated. The difficulty in obtaining accurate values for the gradients madethis method unreliable.

2. Integral or "work done" method.

Figure 44a represents a curve of equation:-

$$E = kL^b$$

(the term c is ignored at this stage, but will be calculated later and used to modify the results of successive iterations).

The work done in extending the specimen between two loads L1 and L2 corresponds to the shaded portion (area A)

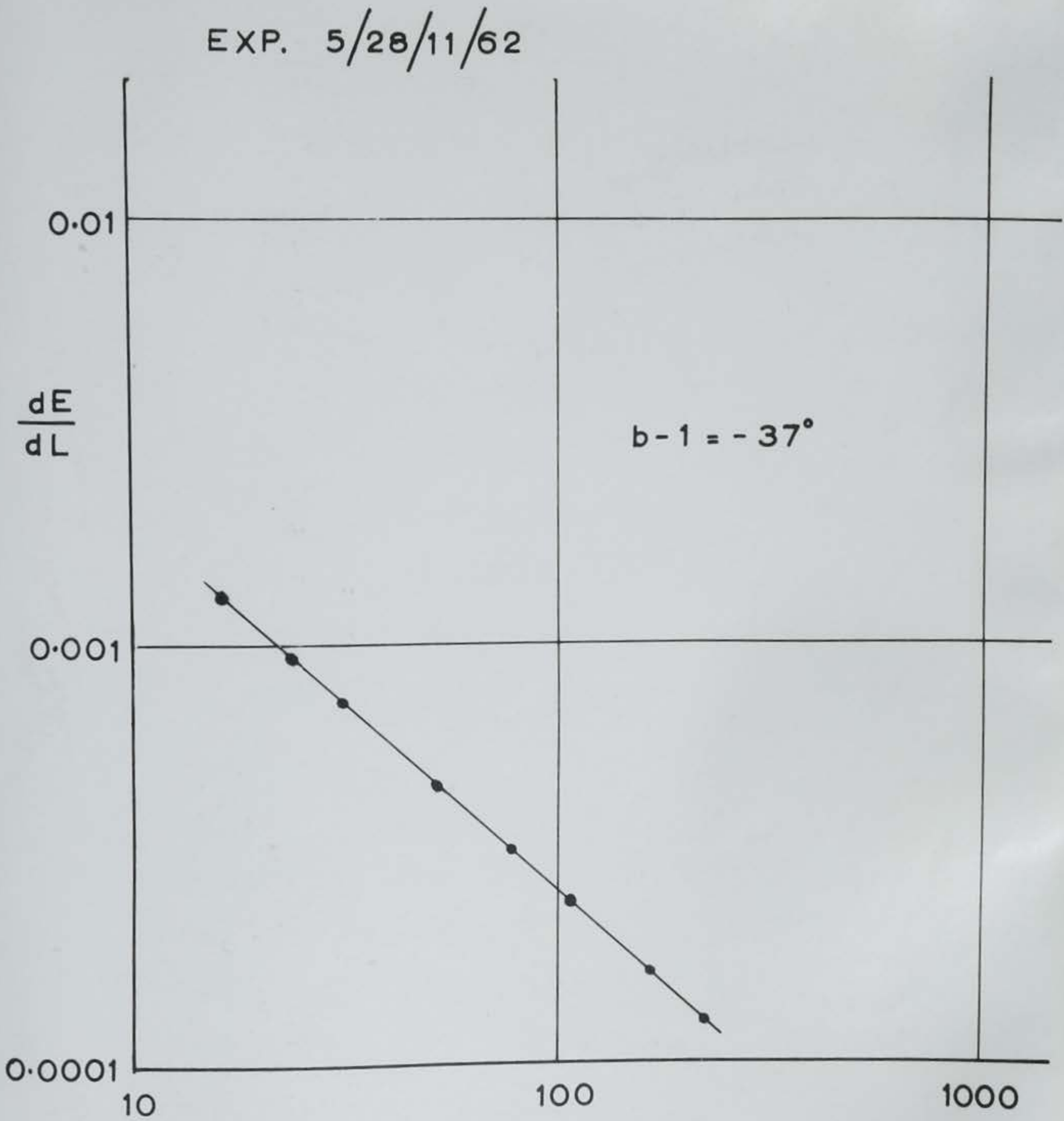


FIG. 43

DIFFERENTIAL METHOD

GRAPH OF LOG $\frac{dE}{dL}$ / LOG L

under the curve and is found by integrating the above equation.

$$\text{Work done} = \int E \, dL$$

$$= k\int L^b \, dL$$

$$\text{Work done between } L_1 \text{ and } L_2 = K \int_{L_1}^{L_2} L^b \, dL$$

$$= K\left[\frac{L_2^{b+1}}{b+1} - \frac{L_1^{b+1}}{b+1}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Similarly in figure 44b it can be seen that work done in loading a specimen from L3 to L4 (area B) is

$$\text{Work done} = k\,\frac{L_4^{\,b+1}}{b+1} - \frac{L_3^{\,b+1}}{b+1} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Eliminating k from equations (1) and (2)

$$\frac{A}{\dfrac{L_2^{\,b+1} - L_1^{\,b+1}}{b+1}} = \frac{B}{\dfrac{L_4^{\,b+1} - L_3^{\,b+1}}{b+1}}$$

$$\frac{A}{B} = \frac{L_2^{\,b+1} - L_1^{\,b+1}}{L_4^{\,b+1} - L_3^{\,b+1}}$$

The quotient of the two area A and B will be known as the "J" function and is seen to involve four loads to the power of b + 1.

Having decided suitable values for L1, L2, L3 and L4, a graph was plotted of the J function over a range of

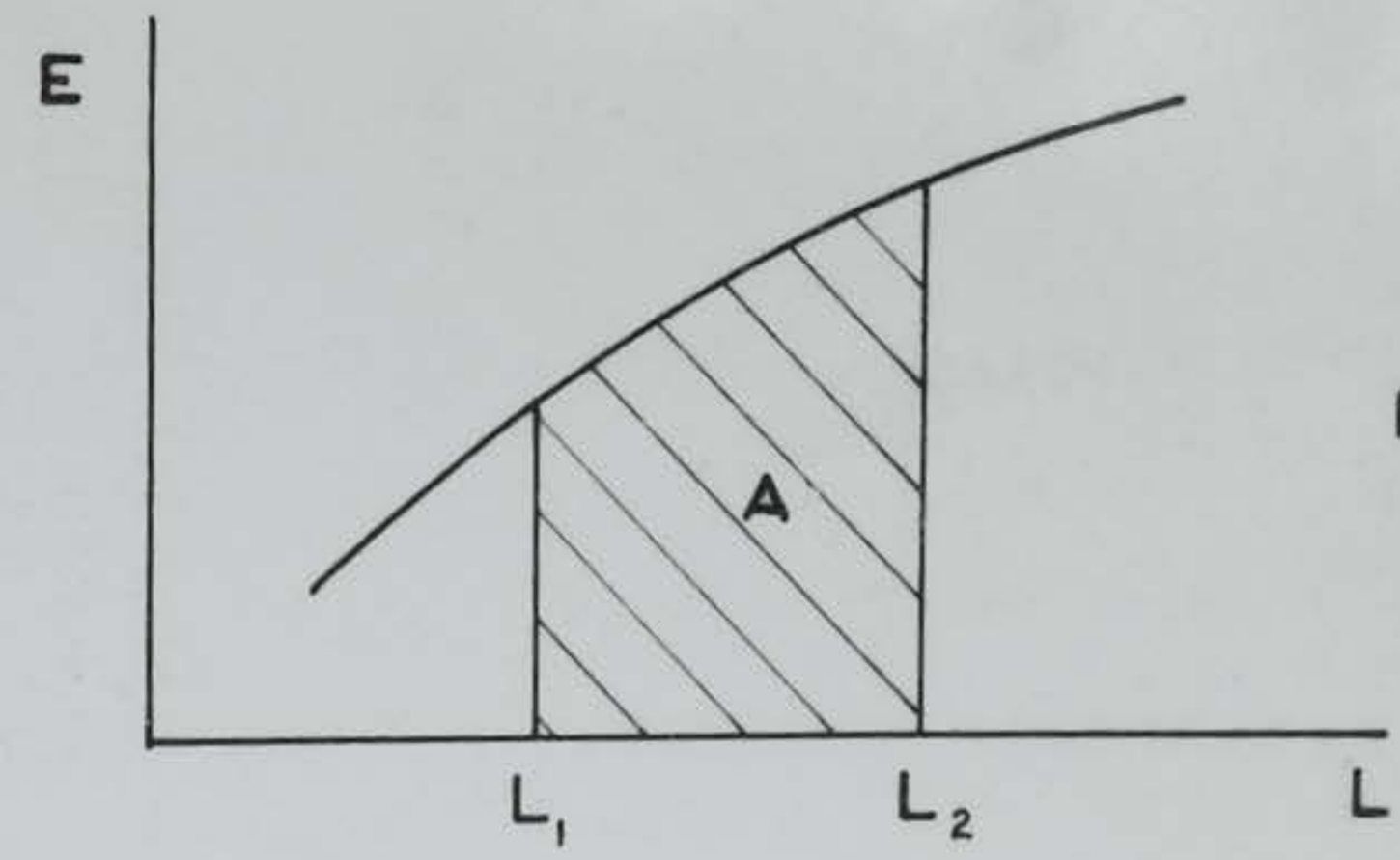


FIG. 44a

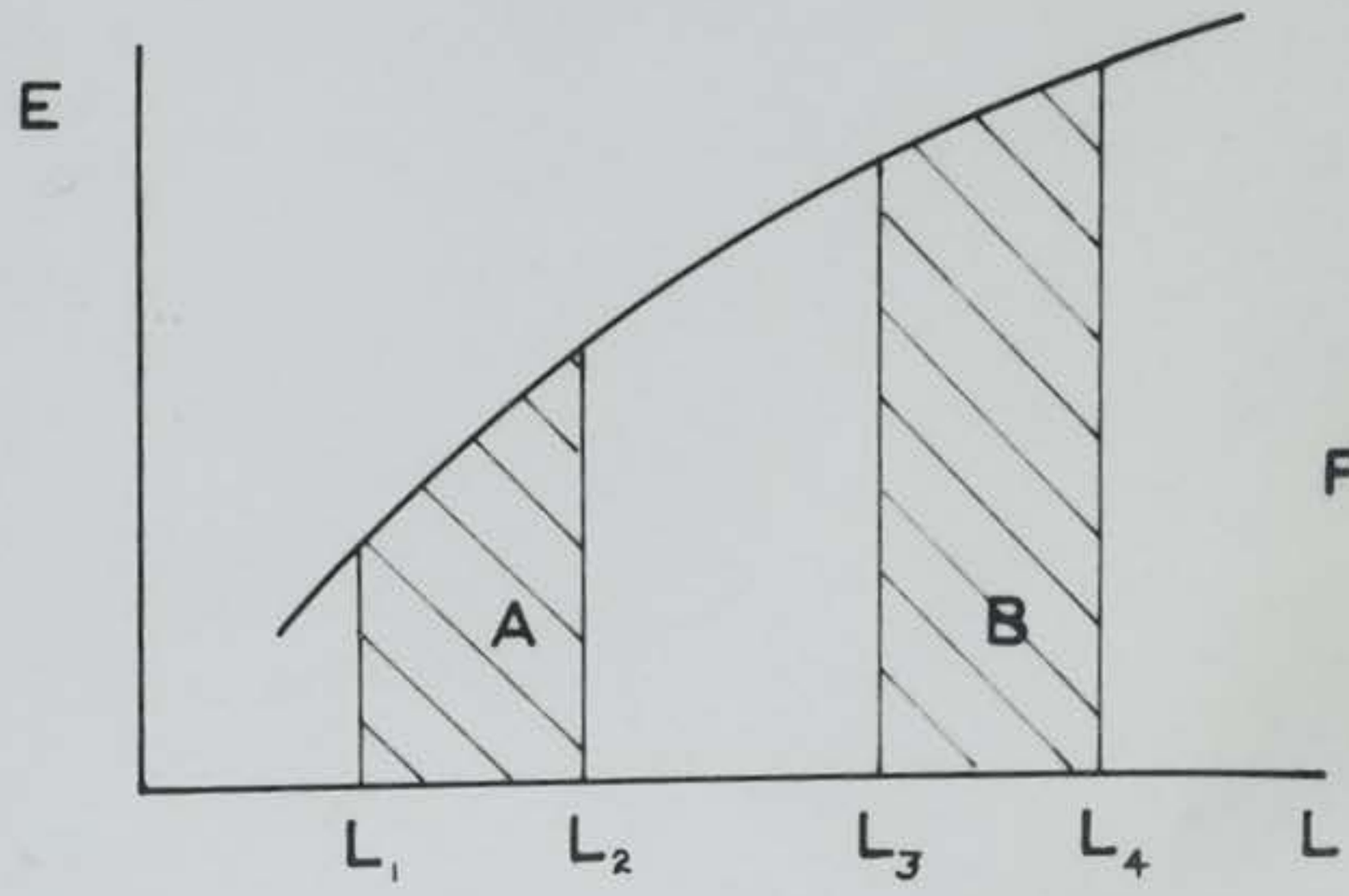


FIG. 44b

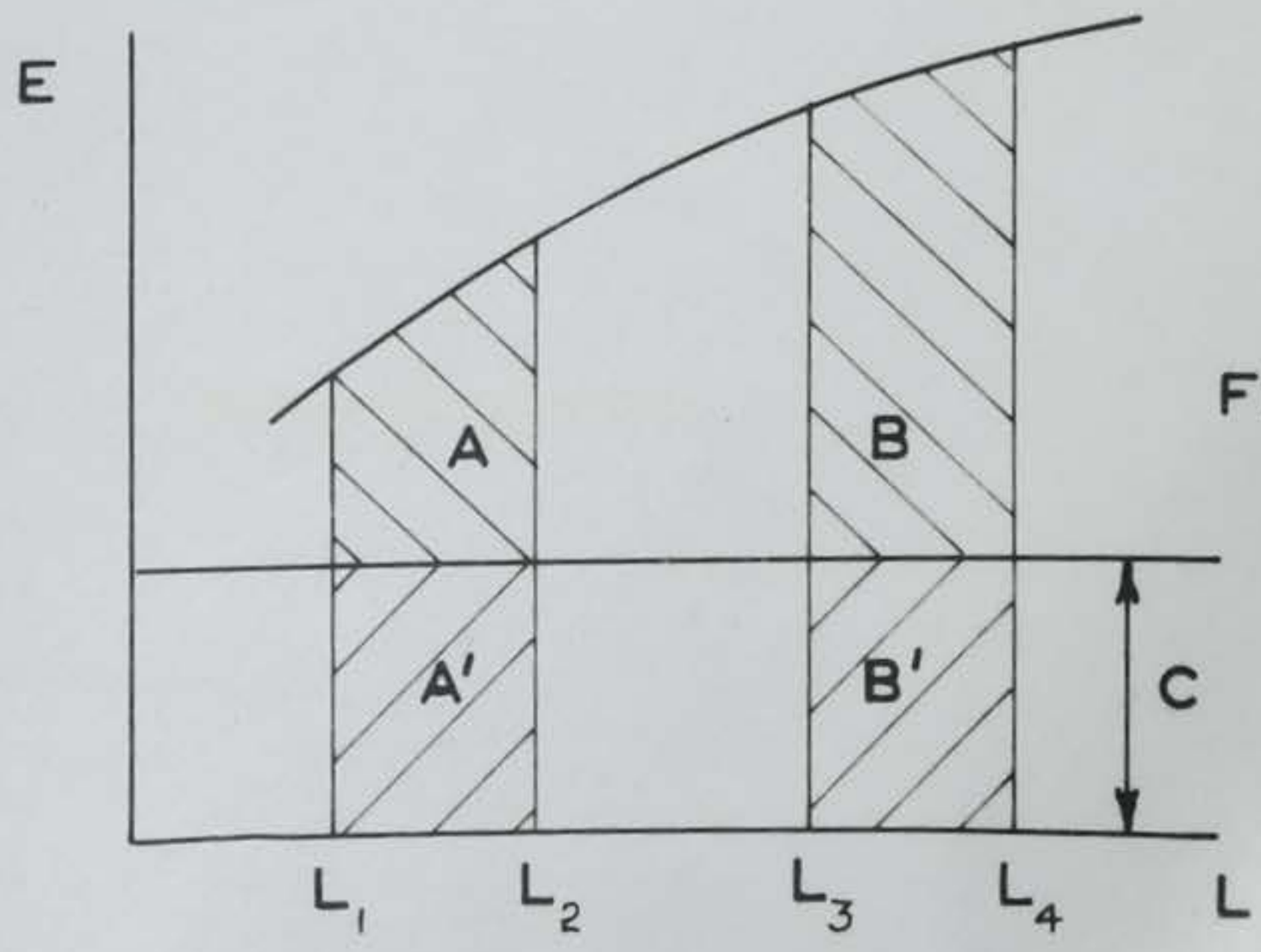


FIG. 44c

DETAILS OF "WORK DONE" METHOD

values of b. The areas A and B corresponding to the selected loads were measured from the stress strain curves with a planimeter and divided to give the J function which is used to obtain the value of b from the b/J function curves.

Having now found b we may calculate the value c, selecting two loads L1 and L2, and from the original equation we obtain:-

$$E_1 = c + kL_1^b$$

and

$$E_2 = c + kL_2^b$$

eliminate k $\quad \frac{E_1 - c}{L_1^b} = \frac{E_2 - c}{L_2^b}$

$$c = \frac{E_1 - E_2\left(\frac{L_1}{L_2}\right)^b}{1 - \left(\frac{L_1}{L_2}\right)^b}$$

This value of c will modify the areas A and B by A' and B' since it represents a shift in the x axis (figure 44c).

$$A' = (L_2 - L_1)c$$

$$A' = (L_4 - L_3)c$$

Thus the new value of the J function $= \frac{A + A'}{B + B'}$

A new value for b can now be obtained from the b/J function curve and hence a second value for c can be calculated. The system usually settled down after about three such iterations and gave values of within approximately ± 2%.

Having found the 'correct' values for b and c, k can be calculated from the original equation.

This method had two disadvantages. Firstly, the curves were in a number of portions, making it difficult to obtain suitable areas. Secondly, difficulty was experienced in calculating the J functions for the b/J function curves with sufficient accuracy. A "linearisation" method was therefore investigated.

3. "Linearisation" method.

When the values of the stress strain curves were plotted on double log paper, a curve was produced rather than a straight line due to the fact that the ordinates were measured from a line below the true axis (figure 45).

This would have been a straight line had the false load axis coincided with the true one. Thus, if the curve was shifted upwards by an amount on the extension axis corresponding to c, the difference between the true and false load axis, it would become linear.

Tests were carried out using 15 points on the curve

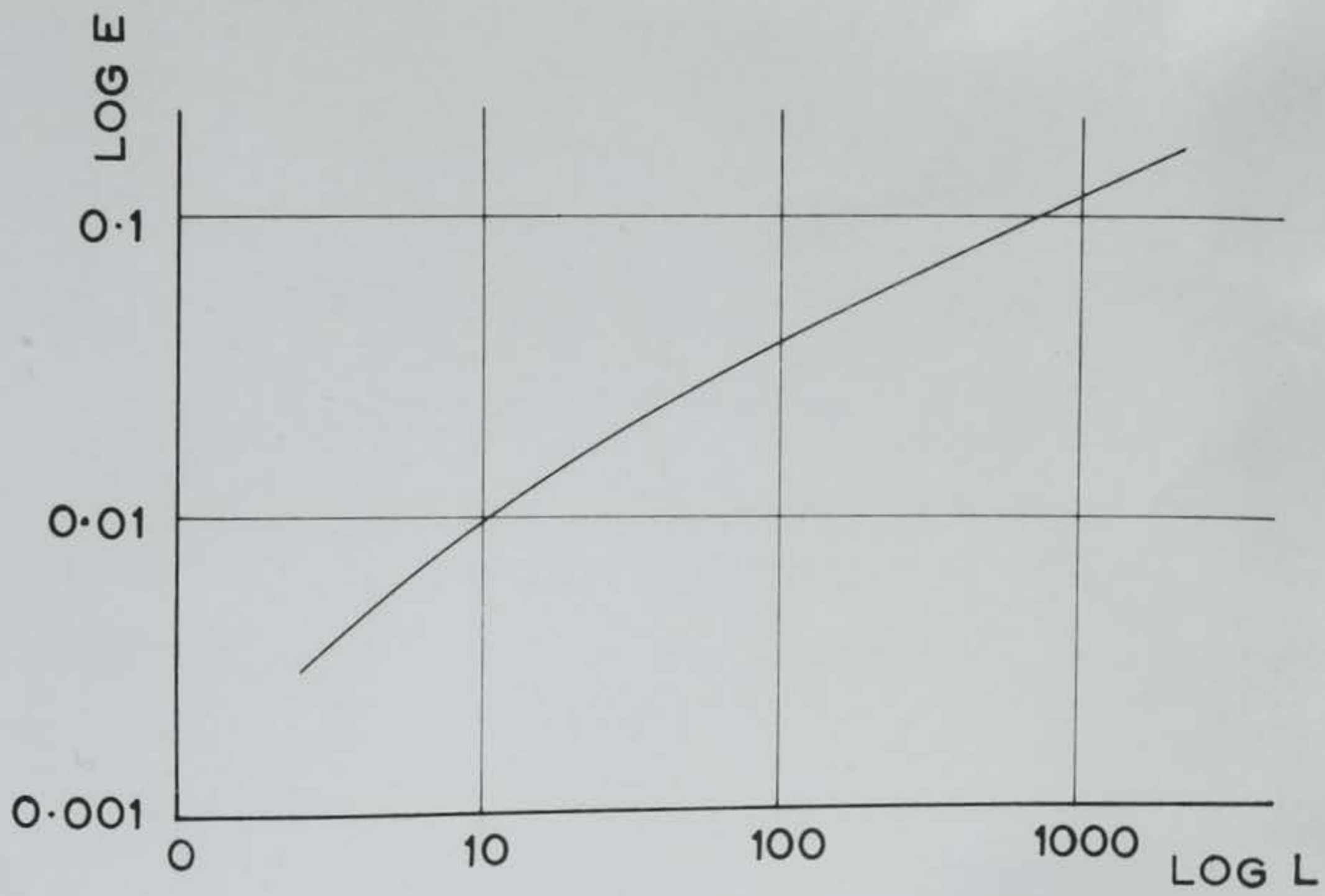


FIG. 45

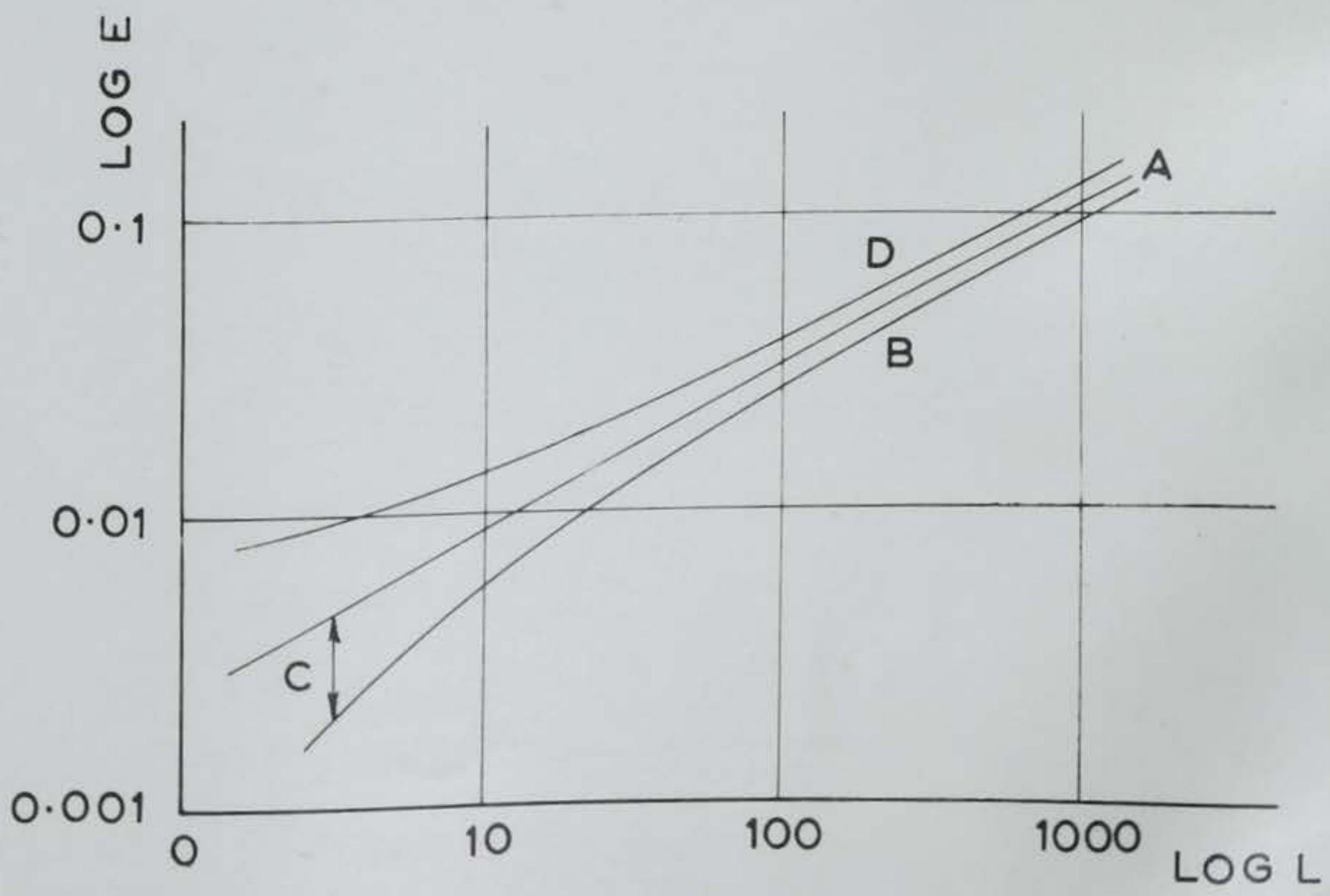


FIG. 46

" LINEARISATION" METHOD

and increasing their ordinates by small increments until a straight line was produced. The total shift corresponded to the value c and the gradient gave the value of b. Figure 46 shows a curve B plotted on double log paper. This corresponded to a stress strain curve with reference to the false load axis. Line A shows the same results after the ordinates had been increased by an amount c. When the process was continued beyond the point of linearisation the line began to curve upwards (curve D). This enabled the optimum position of the line to be deduced. Once the correct line had been found, the constants could be found from the gradient and intercept.

This method had the disadvantages of being extremely tedious and necessitating the use of prohibitively large sheets of graph paper in order to obtain reasonable accuracy. Therefore the technique of linearisation of the curve by a shift process was investigated from an arithmetic rather than a geometric point of view.

4. Arithmetic linearisation method.

The method depended upon the fact that for two or more equal divisions on the load axis the corresponding increase in the extension value would be the same for each division, if the graph was linear.

Equal divisions on the load axis refer to the log load axis and therefore in terms of actual loads the increments were not equal and were governed by the equation:-

$$L = F\, e^{d} \quad \text{.......................................}(1)$$

where L = load value
F = lowest required load, e.g. 100 grams
e = a number determining the number of load values required between two limits e.g. 100 and 1000 grams (a value of e of 1.14 will give 18)
d = 0, 1, 2, 3 ....h, h being the number of values.

Thus, when we took the logarithms of the extensions at the loads calculated from equation (1) and subtracted from each value the previous value, the set of differences were all equal if the extensions had been measured from the correct data. If this was not so, a small increment such as 0.01, was added to each extension value prior to logging and the process repeated until the differences became similar. At this point the sum of the increments which had been added to a particular extension gave the value c.b was found by subtracting the smallest logged extension from the largest and dividing by the difference between the logs of the corresponding loads.

This method was also tedious and the necessary calculations were done on the electronic computer. The system

was the same as above except that the mean of the differences was calculated and thence the deviation of the difference from the mean value. A value x, the square root of the sum of the squares of the deviations was calculated. For the correct result x should be a minimum. Thus the process of adding 0.01 to the extension and working out the corresponding values of x was continued until the difference between a value of x and its previous value became negative, signifying that the minimum had been passed. 0.02 was then subtracted from the increment total (to ensure that this value was before the minimum) and the process continued by adding increments of 0.001 until the minimum was again found. This gave values for the constants in the equation and could be computed in approximately three minutes. This method worked well but did not give the high accuracy of a method developed later, based on a Taylor's series expansion.

5. Taylor's series expansion method.

A suitable curve fitting method for this problem was proposed by Steven's (1951). The analysis was based on the Taylor's series expansion in the non-linear variable b.

Taylor's theorem states that if x and H be two independent quantities, and if f (x and H) (i.e. the function

of x and H) is to be expanded in a power series in H about the point x then:-

$$f(x + H) = f(x) + Hf'(x) + \frac{H^2}{2!} f''(x) .. \frac{H^h}{h!} f^h(x)$$

where f' is the first derivative of the function and f'' the second etc. We may now expand our equation according to Taylor's theorem about b = bo and assume that terms involving the second derivative and higher are negligible. (Let Log e = ln)

$$E = c + kL^b$$

$$F(x + H) = f H + x f'(H)$$

$$E = c + kL^{bo} + k(b-bo)\ lh\ L.D^{bo} \quad ....(1)$$

For simplification the following substitution will be made:-

$$u = L^{bo} = \exp [bo\ lh\ L] \quad .....(2)$$

$$v = lh\ L \exp [bo\ lh\ L] \quad .....(3)$$

$$d = k(b - bo)$$

and hence equation (1) becomes

$$E = c + ku + dv \quad .....(4)$$

Given a set of n points Ei, Li, it is required to determine the coefficients c, k and d such that equation (4) fits the points in a least square sense, that is, such that $\sum_{i=1}^{h} [E - c - kui - dui]^2$ is a minimum. This implies that the partial derivatives of this sum with respect to k, c and d are zero.

EXPANDING :-

$$h(c)^2 + \sum E_i^2 + \sum (d v_i)^2 + \sum (k u_i)^2 - 2c\sum E_i - 2k\sum E_i u_i$$

$$+ 2ck\sum u_i - 2d\sum E_i v_i + 2dc\sum v_i + 2dk\sum u_i v_i$$

$\therefore \frac{\partial}{\partial a} = 0$ GIVES :-

$$2hc - 2\sum E_i + 2d\sum v_i + 2k\sum u_i = 0$$

$$\therefore \underline{\sum E_i = ch + k\sum u_i + d\sum v_i} \quad \cdots (5)$$

$\frac{\partial}{\partial k} = 0$ GIVES :-

$$2k\sum u_i^2 - 2\sum E_i u_i + 2c\sum u_i + 2d\sum u_i v_i = 0$$

$$\therefore \underline{\sum E_i u_i = k\sum u_i^2 + c\sum u_i + d\sum u_i v_i} \quad \cdots (6)$$

$\frac{\partial}{\partial d} = 0$ GIVES :-

$$2d\sum v_i^2 - 2\sum E_i v_i + 2c\sum v_i + 2k\sum v_i u_i = 0$$

$$\therefore \underline{\sum E_i v_i = d\sum v_i^2 + c\sum v_i + k\sum v_i u_i} \quad \cdots (7)$$

FROM (5) SUBSTITUTE FOR C IN (6) AND (7)

$$k\left[\sum u_i^2 - \frac{\left(\sum u_i\right)^2}{h}\right] + d\left[\frac{\sum u_i \sum v_i}{h} - \frac{\sum u_i \sum v_i}{h}\right] = \sum u_i E_i - \frac{\sum u_i E_i}{h} \quad \ldots (8)$$

$$k\left[\sum u_i v_i - \frac{\sum u_i \sum v_i}{h}\right] - d\left[\sum v_i^2 - \frac{\sum v_i^2}{h}\right] = \sum v_i E_i - \frac{\sum v_i E_i}{h} \quad \ldots (9)$$

NOW LET

$$\alpha \equiv \sum u_i^2 + \left(\frac{\sum u_i}{h}\right)^2$$

$$\beta \equiv \sum u_i v_i - \frac{\sum u_i \sum v_i}{h}$$

$$\rho \equiv \sum v_i^2 - \frac{\left(\sum v_i\right)^2}{h}$$

$$\phi \equiv \sum u_i E_i - \frac{\sum u_i \sum E_i}{h}$$

$$\Omega \equiv \sum v_i E_i - \frac{\sum v_i \sum E_i}{h}$$

NOW SUBSTITUTE IN (8) AND (9) AND SOLVE FOR k AND d

$$\alpha k + \beta d = \phi \quad \ldots (10)$$

$$\beta k + \rho d = \Omega \quad \ldots (11)$$

$$(\beta^2 - \alpha\rho)d = \phi\beta - \Omega\alpha$$

$$\therefore \quad \alpha = \frac{\phi\beta - \Omega\alpha}{\beta^2 - \rho\alpha}$$

SUBSTITUTE IN (10)

$$\alpha k + \frac{(\beta^2\phi - \Omega\alpha\beta)}{(\beta^2 - \rho\alpha)} = \phi$$

$$\therefore \quad k = \frac{1}{\alpha}\left(\phi - \left(\frac{\beta^2\phi - \Omega\alpha\beta}{(\beta^2 - \rho\alpha)}\right)\right)$$

Now $\quad d = k(b - b_0)$

$$\therefore \quad b = \frac{d}{k} + b_0$$

FROM (5)

$$a = \frac{\sum E_i - k\sum u_i - d\sum v_i}{h}$$

Thus expressions have been derived to give the values of the constants c, k and b in the stress strain equation.

This method was now used as follows:- an estimate

of the final value of b was made and denoted as bo; this was used in equations (2) and (3) and thence the whole procedure carried out until b had been found. However, since the second and higher order terms omitted in the Taylor's series expansion may not be negligible, these equations may not give sufficiently precise estimations of the constants c, k and b. The value of b thus determined would however be an improved estimate.

If this value of b now became the new bo it could be used to calculate an even better value of b. This process is called iteration and may be carried out any number of times. The value of b became more accurate with each iteration and the process was used until the required precision was achieved. The total number of iterations naturally depended on how near the initial estimate of bo was to the true value.

Having arrived at a satisfactory value for b the values of the other constants could be deduced from the above equations.

The magnitude of this procedure was such that it would take several days to calculate manually the values of the constants for one piece of skin with any accuracy. However by using an electronic computer the time was reduced to one or two minutes.

The computer.

The computer used was a Ferranti Pegasus which had a large memory consisting of several thousand locations designated v0, vl, v2, v3 ....vn, and termed variables, into which the values for the data were placed. Thus if an instruction on the programme was

vl + v2 = v6

the sum of the numbers in vl and v2 were placed in v6. There was a second set of locations in the memory n0, nl, n2 ....nn termed indices which were used mainly for counting and organising the programme. Thus by the use of variables and indices long and complicated single instructions could be given in the programme which were carried out in a single step by the computer, e.g.

$$v400 = v(309 + n4 - n3) \times \mathrm{LOG}\left(v\,39 \times v\,(100+n4)\,/\,4\atop (91 - n3)\right)$$

The programming system used was extended Autocode (Pegasus Autocode, 1958) and represented a reasonably rapid means of producing a workable programme.

All data read into the computer was in the form of paper tape which had holes punched in it according to a code to represent the various characters. Such tape was prepared on a punch, which was similar to a typewriter, and could be fed into the computer. The calculated

results from the computer were also in the form of punched tape and could be converted to a printed output by means of a teleprinter.

Programme details.

A flow diagram was produced before actually writing the programme. This diagram gave the main stages of the calculation and indicated the way in which they were interrelated, thus providing a rapid visual guide to the programming and the subsequent work.

Referring to the flow diagram (appendix 3) the first instruction consisted of reading into the computer a number representing the total number of specimens to be tested and a second number h equivalent to the number of points taken from the stress strain curve. The details of the computer programme are shown in appendix 4. An assumed value for b, i.e. b_o was now read in, preceded by h load values which were stored in the memory prior to having their logarithms calculated and stored.

A series of calculations now followed which resulted in the values of α β C being found and stored. These values were only used for the first iteration of a particular test, subsequent values involving an improved b were calculated and stored elsewhere. The specimen

number was now read in followed by h extension values and from these and the previous results a new value of b was found which was subtracted from the original estimate bo. If this difference was found to be greater than 0.01 the new value of b was substituted for bo and the iteration carried out using an alternative route. This was for two reasons, firstly one did not wish to erase the values required for subsequent calculations and secondly it was important not to read in the next set of extension values at this stage, but to use the ones of the previous iteration.

Iteration continued until an acceptable value of b was obtained whence the other constants were calculated and printed out. The errors between the actual and calculated extensions were also calculated and printed out against their corresponding loads.

A test was now carried out to check if this was the last set of data in the series in which event the computer was made to stop. If this was not the case, the next set of extension values was read in. For the first iteration the original bo was used and the stored values of previous first iterations were used without need for further calculation. Once the initial value of b had been calculated

for this test the procedure was as described above.

The programme was arranged so that it was capable of handling a maximum value of h of 40. In practice it was found that 15 points were adequate to give the required accuracy.

Effects of errors.

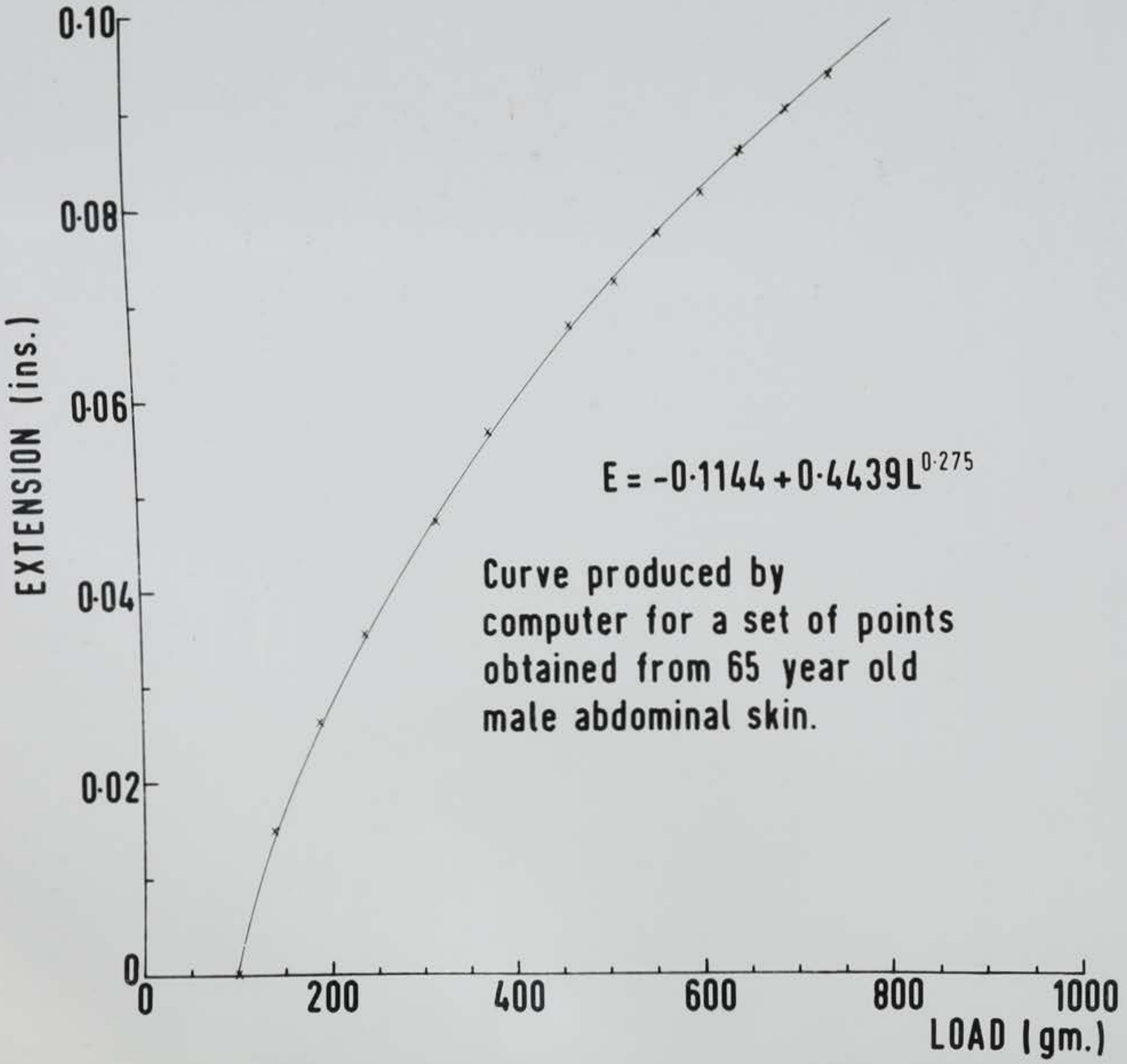
It was found desirable after the values of the constants had been found to use these to calculate values for the extensions at the corresponding loads and compare these with the original extensions obtained from the stress strain curves. A difference of less than 0.001 was found to be of no consequence, this being greater than any measuring inaccuracies. This provided a check on the quality of the 'fit' obtained by the computer, and showed if mistakes had been made in the extension measurements and also gave an indication of the yielding of the specimen (a phenomenon which was difficult to detect at an early stage).

The effectiveness of this method is demonstrated in plate 24 which illustrates a typical result. The curve was given by the computer and the superimposed crosses were the values obtained from the stress strain curve.

Tests were also carried out to investigate the effect of introducing errors of different magnitudes to

PLATE 24. A curve produced by the computer superimposed on experimental results obtained from a stress strain curve of skin. Note the effectiveness of the fit, and the point of inflection can be seen at about 510 gm.

EXP. 302


0·10
0·08
0·06
0·04
0·02
0
EXTENSION (ins.)
0
200
400
600
800
1000
LOAD (gm.)
E = -0·1144 + 0·4439L^0·275
Curve produced by computer for a set of points obtained from 65 year old male abdominal skin.

various parts of the curves. It was found that a given error produced a greater effect at the beginning or end of a curve than in the middle.

The programme was arranged so that it continued iterating until the value of b differed from its previous value by less than 0.01. As the function was found to converge extremely rapidly this represented an exceptionally accurate result, an error much less than 1%.

E. MINIMISATION OF SCATTER OF RESULTS

It was observed that the results obtained from specimens prepared under identical conditions and taken from adjacent areas of the same skin sample showed wide variations. Such variations could be attributed to three sources of error:-

1) Gross lack of homogeniety throughout the skin structure manifest even in areas of a few inches extent.

2) Variations in the preparation and mounting techniques of the sample.

3) Inconsistencies in the curve fitting techniques.

These three sources of error were dealt with systematically in the following manner:-

1) Even if the fibrillar structure of a piece of skin is heterogenious it should consist of the same type of fibre within atleast a small given area. Thus it would be expected that the values of the index b would remain constant, since, according to the previously derived theory b depends only on the quality of the collagen and not the quantity. Collagen analysis of skin showed that the composition varied by only a few per cent and was too small to account for the observed variations. However b did vary and therefore the influence of other factors was investigated.

It is conceivable that the fibre structure of skin is large compared with the cross sectional area of the specimen (figure 47a). In this case a specimen of the size used may cut across the structure in such a way that fibre ends emerging at the sides of the specimen may be pulled free and slide over one another, there not being enough "weave" to hold the fibres in position. If the specimen is large compared with the fibre structure (figure 47b) this is unlikely to occur. Investigations were carried out to determine whether the structure size was indeed influencing the results.

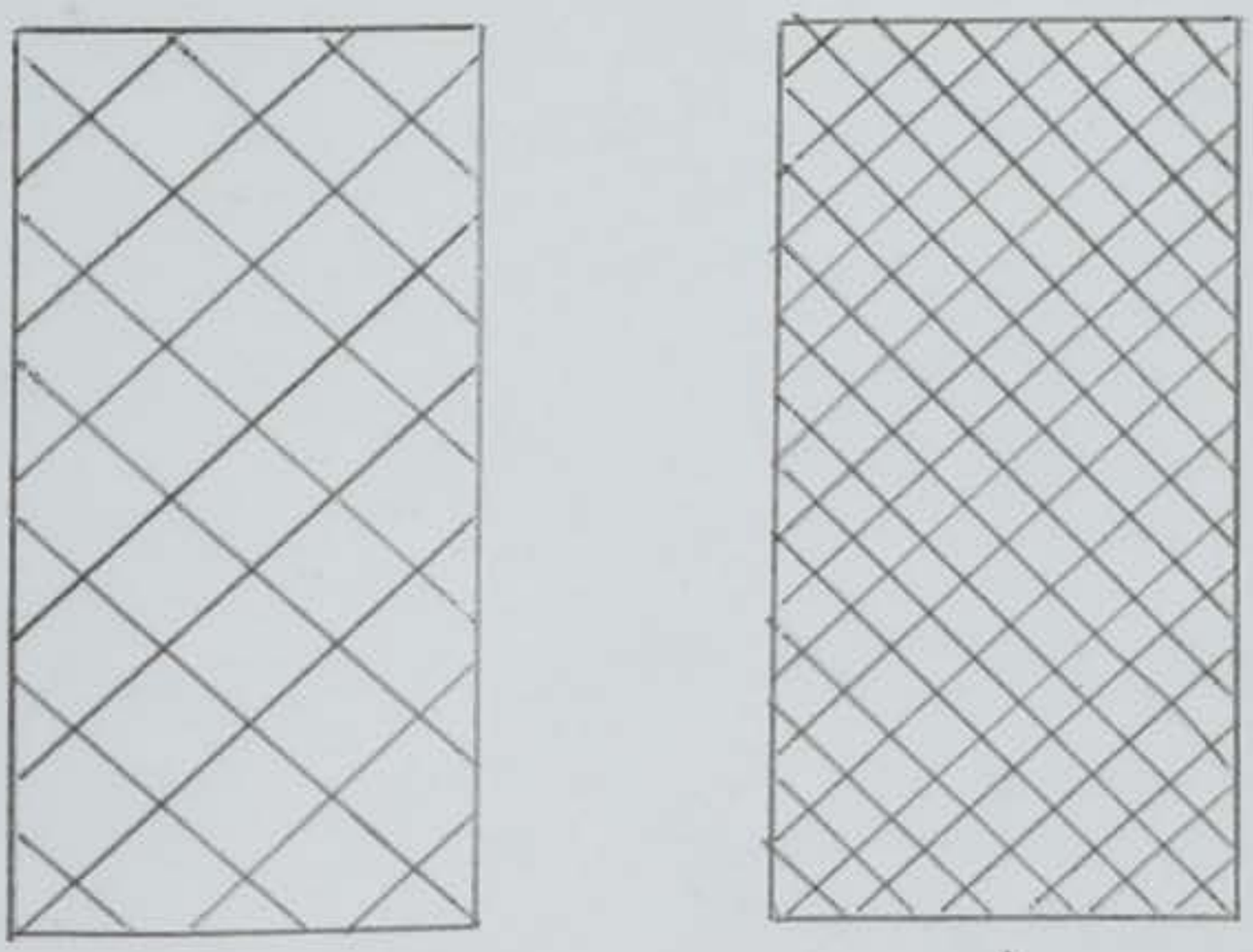


FIG. 47

Experiments were performed involving two large pieces of skin, each cut into 24 specimens. Twelve specimens from each group were cut in the normal way and twelve were cut to the same thickness but twice the width (by increasing the spacing between the blades of the die). The specimens were tested on the extensometer and the results calculated in the usual way. If the structure size was in fact causing the observed scatter, a wider specimen should have overcome this problem to some extent, and produced a reduction in scatter. This however was not found to be the case, and it was concluded that the variations were probably due to some factor other than the heterogeniety or large fibre structure of the specimen.

Great care was taken throughout to minimise the variations caused by preparation and mounting techniques. However in an attempt to determine whether the variations in results were due to such causes, experiments were made on large numbers of specimens cut from the same piece of skin in which extra precautions were taken.

The introduction of the slab-cutting technique produced specimens with a high degree of dimensional reproducibility. Extreme care was exercised in the mounting and testing of specimens, but such efforts did nothing to reduce the scatter of results.

3) Further investigation showed that results from stress strain curves were extremely sensitive to slight changes in mathematical techniques. This was therefore studied further as detailed below.

1. Curve fitting improvements.

Initial curve fitting work was carried out on the computer using 15 ordinates between a 100 and 1000 grams. Having calculated the constants for the curve, the computer was made to calculate the true ordinates for each load from these constants. The ordinate as measured was then subtracted from the calculated value, and the difference or error printed out against the appropriate load (table 7). The original purpose of this device was to ensure that a good "fit" had been achieved by ensuring that differences never exceeded some arbritary value. However observation revealed that the magnitude and sign changes of these differences followed a distinct pattern.

The most usual form of the difference distribution was such that the first one or two values were positive, followed by a series of about six negative values, reaching a peak and diminishing again. A series of about six positive values then occured, similarly reaching a peak and diminishing again, and finally the last two or three

7

+273	+19	−0.553	+0.9167	+0.1042	+2
+0.0100	+0.000927				
+0.0148	−0.000613				
+0.0196	−0.000056				
+0.0244	−0.001103				
+0.0293	−0.000914				
+0.0342	−0.000104				
+0.0391	+0.000758				
+0.0415	+0.000131				
+0.0460	+0.000029				
+0.0509	+0.000783				
+0.0557	+0.000926				
+0.0602	+0.000443				
+0.0648	+0.000088				
+0.0697	+0.000165				
+0.0742	+0.000213				
+0.0787	+0.000116				
+0.0833	−0.000007				
+0.0879	−0.000532				
+0.0927	−0.000878				

TABLE 7.

DIFFERENCE DISTRIBUTION

values were negative. The implication of this can best be appreciated by reference to figure 47 which shows a magnified picture of the situation (it should be noted that the magnitude of the difference is of the order of 0.1 per cent of the ordinate, i.e. at the limit of direct measurement). The simple curve represents the curve as calculated by the computer and the superimposed "wavy" curve the results actually derived from the extensometer. It is seen that the experimental curve is sigmoid in shape (i.e. there is a point of inflection along its length), although this was not revealed until arithmetic comparison was made with a curve of the correct shape. Thus the proposed equation cannot be expected to give a good fit over the full range. Examination of the difference tables revealed that the point of inflection occurred at a different place even for similar specimens and was thus related in some way to the general variation of the results.

To investigate this variation in the position of the point of inflection a number of similar specimens were prepared and tested on the extensometer to a load of 2,000 grams, i.e. twice the normal value. All specimens were found to yield somewhere between 1000 and 2000 grams, although visual inspection of the portion of the curve up

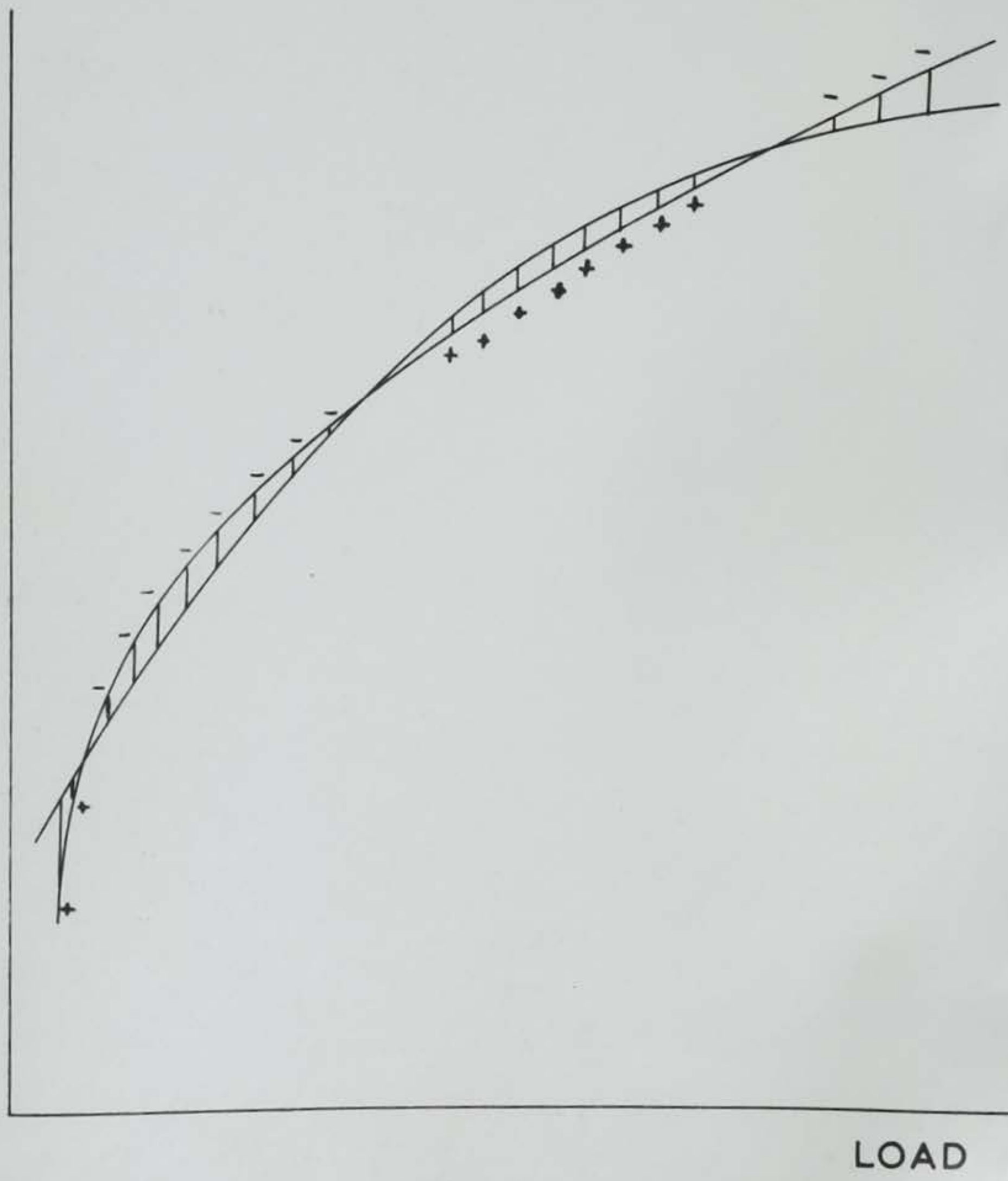


FIG. 47

COMPUTED & ACTUAL CURVES

to 1000 grams gave no indication of the time yielding actually occurred. The constants for the skin were then calculated using the normal range of 100 to 1000 grams, and it was found that in the cases where the point of inflection occurred early in the curve the specimens had yielded early. Conversely, a point of inflection high up the curve indicated late yielding. Further investigations revealed that the specimens which yielded early gave a large value for b and a small value for k and vice versa. It was thus concluded that the scatter in the results was dependent wholly or partially on the point at which the specimens would yield, and that the influence of the yield point extended far down the curve, certainly to a point a little higher than the point of inflection.

The portion of the curve used extended not from zero load, but from a point where it could be assumed that the equation changed from a logarithmic to a power function (i.e. that the fibres had become orientated). It was reasoned that the arbitrary starting point of 100 grams may also influence the scatter, as it was observed that the pattern of the first few points in the difference table was not always the same for similar specimens.

Thus it would seem that out of a total range of 100 to 1000 grams there was a variable source of error at

both extremes indicating the need for a narrower range which had to be determined for each specimen in order that the optimum section of the curve might be analysed.

2. Difference distribution.

Before it was possible to select the optimum range for a particular specimen, it was necessary to investigate the properties and influence of various difference distributions. Initially simple cases were examined:-

1) In figure 48a A is a power curve of equation $E = c + kL^b$, B is a similar curve which is more concave upwards. If these curves are considered in the light of the "linearisation method" previously mentioned, it will be seen that the curve B will require a greater vertical shift to linearise than A, because of its greater curvature. This will produce a smaller value of b and larger values of k and c.

2) Figure 48b shows a curve A of the same equation as above and the second curve B less concave upwards. Again by using the linearisation principle it is seen that the curve B will have a greater value of b and a smaller value of k and c than the curve A.

3) Figure 48c shows the actual type of curve obtained, and is seen to be the results of figure 48a and figure 48b superimposed. This situation is obviously a

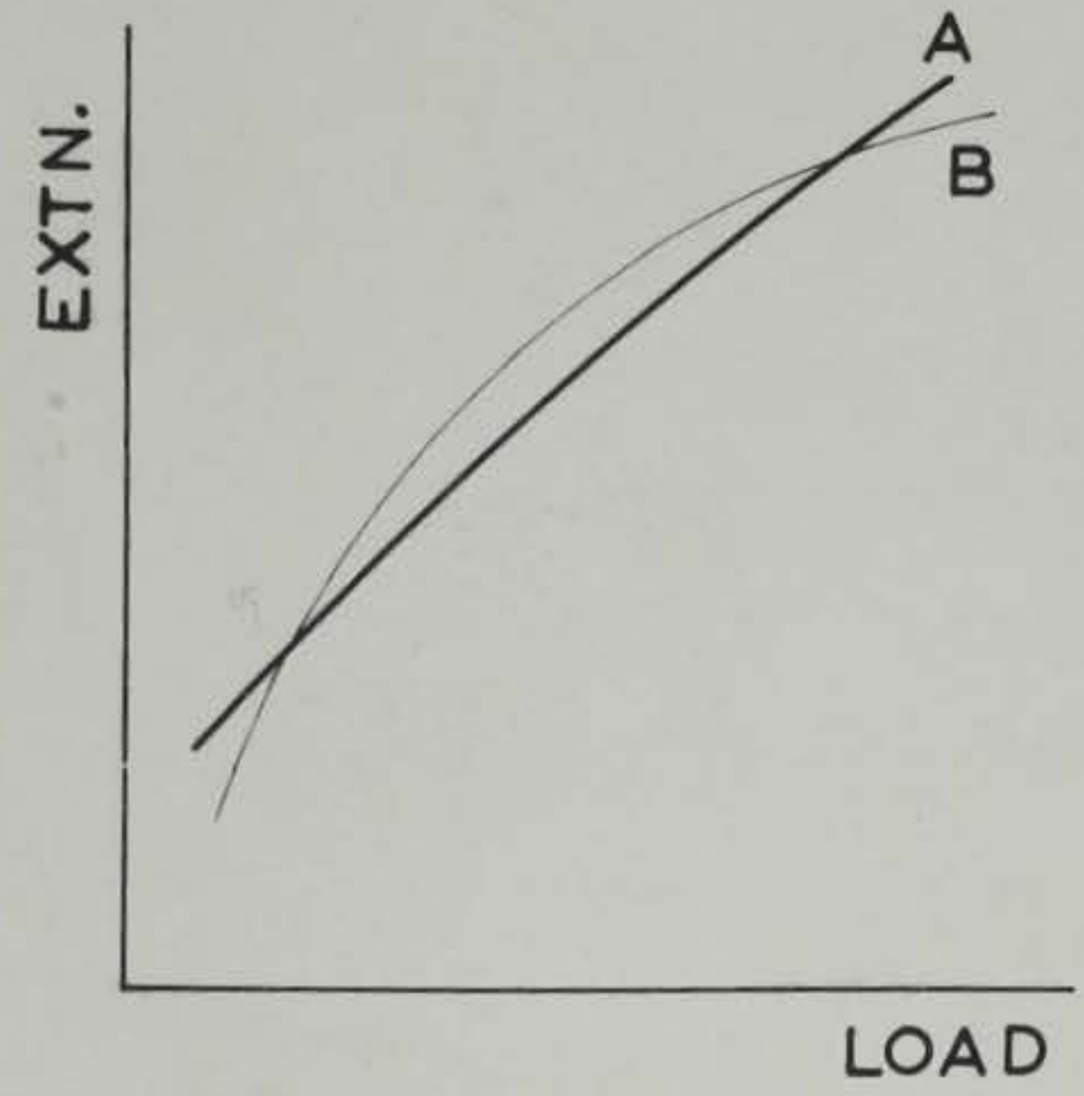


FIG. 48a

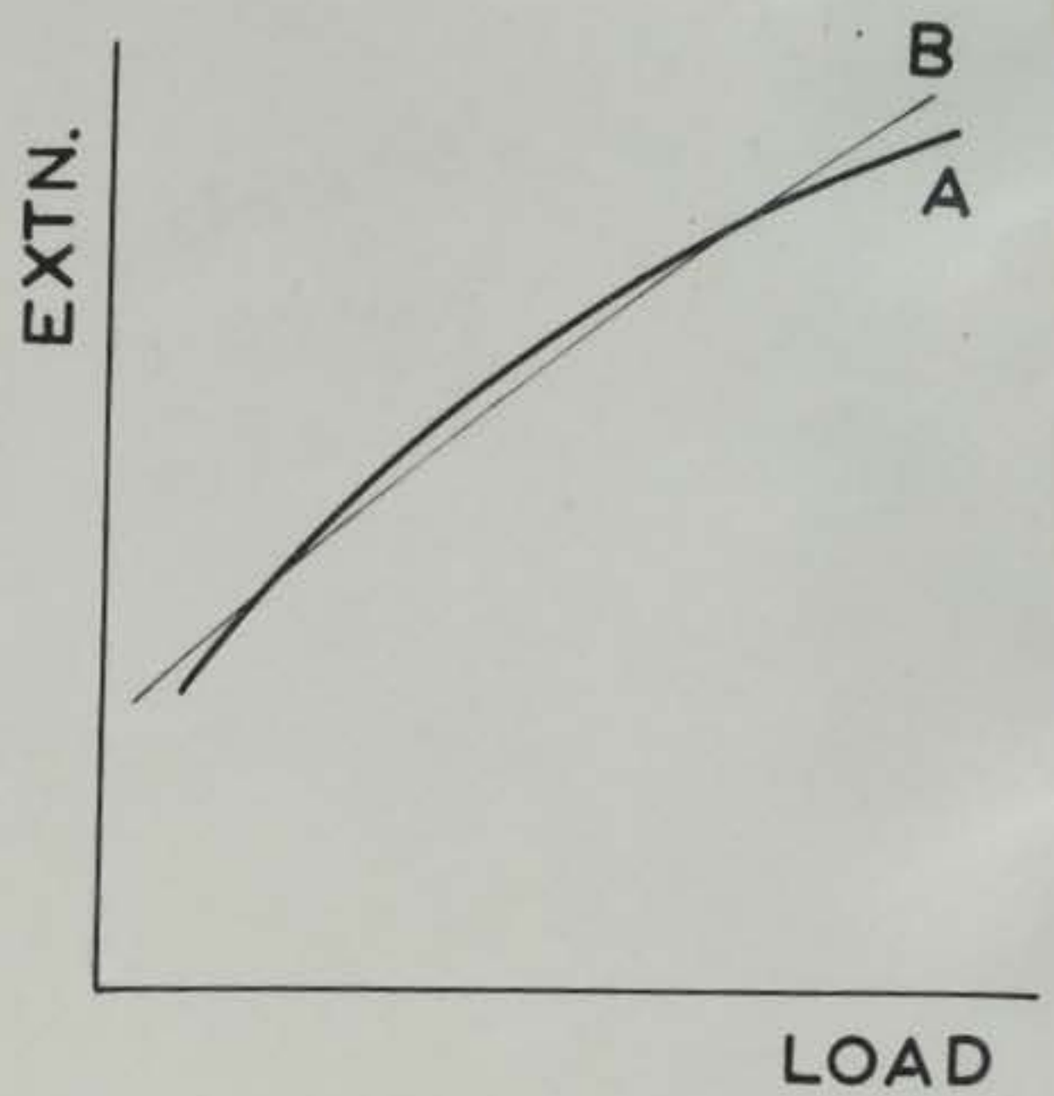


FIG. 48b

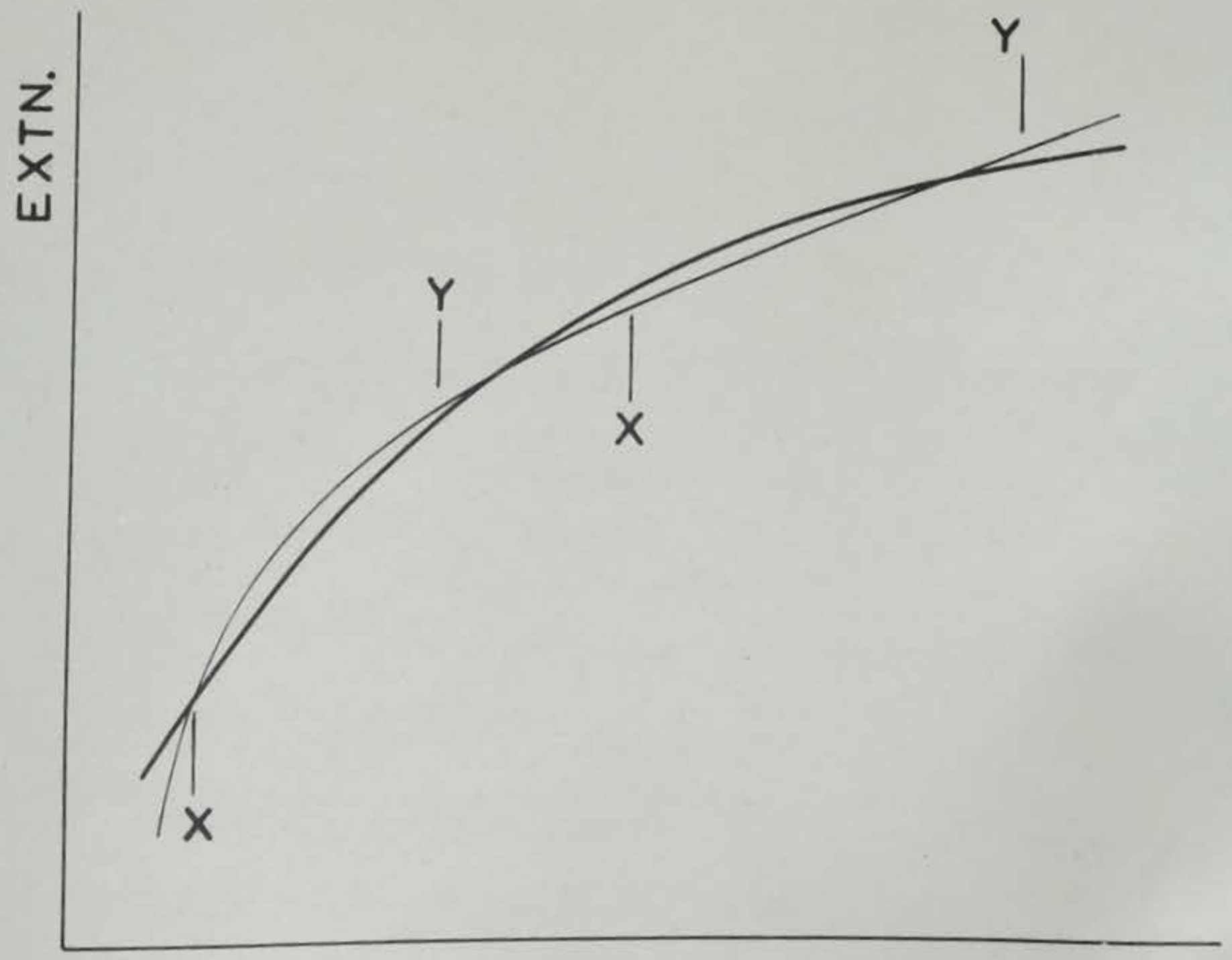


FIG. 48c

RANGE SELECTION

compromise, since it requires two conflicting conditions to be satisfied. However, the lower portion of the curve is found to dominate due to the fact that the intervals on a logarithmic scale diminish as one proceeds along axis.

3. Range selection.

If figure 48c is now assumed to represent the difference distribution of the results of a specimen, it is necessary to decide the actual range over which the assumed equation may be expected to hold. For example the results given for the portion of the curve X - X will give a lower value of b than that of the curve A while the range Y - Y will give a higher one. This was in fact checked and found to be the case for specimens when results for various rnages were calculated on the computer. The problem was now to find the optimum range for any particular specimen according to its differance distribution pattern.

In the light of previous work, it was decided to examine the values of the constants of a specimen over a number of ranges. To improve the accuracy, the number of ordinates was increased from 15 to 19, that is 100 to 1000 grams in 50 gram intervals. The selected ranges are shown in Table 8 in which the third column gives the number of ordinates per range. Six ordinates were considered to be the minimum number to give a reasonable result and eleven

START	FINISH	points/range	
100	1000	19	main range
150	450	7	
	500	8	
	550	9	
	600	10	
	650	11	
200	450	6	
	500	7	
	550	8	
	600	9	
	650	10	
	700	11	
250	500	6	
	550	7	
	600	8	
	650	9	
	700	10	
	750	11	
300	550	6	
	600	7	
	650	8	
	700	9	
	750	10	
	800	11	
350	600	6	
	650	7	
	700	8	
	750	9	
	800	10	
	850	11	

Table 8. The limits of the load ranges for which the rheological constants of skin were calculated.

the maximum practical number which would be handled without the number of ranges becoming prohibitive.

Initially the programme previously mentioned was used. This however involved a preparation of thirty data tapes (i.e. one for each range) and resulted in a total calculating time on the computer of over half an hour per specimen. 40 specimens (i.e. two batches of 20 identical specimens) were treated in this way and the information gained from the results was ample to enable the optimum range system to be evolved. It was assumed that as the specimens were identical the same value of b should be produced each time. Consequently an attempt was made to select a particular range for each specimen which would fulfil this condition. The ranges were selected with reference to the different distributions for the particular specimen. For example the values of b were compared when the ranges were taken between the negative and positive peaks of the distribution curves. Numerous ranges were tried in this way until it was found that there was good agreement in the values of b when the range was taken from the negative peak to the point of inflection. However, for a specimen in which the distribution showed no great irregularity due to curve inconsistencies, several ranges in the vicinity of the one mentioned all gave similar values, suggesting that this was a uniform section of the curve

which could be assumed to be characteristic of the specimen. To minimise the errors caused by slight irregularities in the curve, the mean of seven values, i.e. three each side of the main one was taken.

To overcome the problem of calculating each range for each specimen separately, a new programme was written (appendix 5). The main advantage was that after having beed fed with the 19 ordinates the computer selected the ranges itself and calculated the appropriate constants. Thus all the ranges for one specimen were calculated at the same time, rather than one range for all specimens as with the previous method. This system apart from reducing the required number of tapes from thirty to one per specimen, also worked more quickly on the computer, treating all the ranges for one specimen in eleven minutes.

4. The programme.

Much time was saved with this programme (appendix 5) by arranging for all the results calculated for the various loads to be fed into the computer store and retained there to eliminate the need for calculating the values for each specimen. This necessitated the preparing of a second programme (appendix 6), to calculate this information and to put it on tape so that it could be fed in as data after the main programme. The store allocations of these pre-calculated values is given in Table 9.

v 40	= bo
v 41 - 60	= 19 ext values
v 80 - 98	= 19 load values
v 99	= 0
v 100 - 118	= 19 log load
v 119	= 0
v 120 - 138	= 19 EXP (3 x log load)
v 139	= 0
v 140 - 150	= 19 log load X EXP (3 x log load)
v 159	= 0
v 160	$=\sum u$
v 161	$=\sum v$
v 162	$=\sum v^2$
v 163	$=\sum v^2$
v 164	$=\sum vu$
v 165	$= \alpha$
v 166	$= \beta$
v 167	$= \rho$
v 168	= 0
v 169	= 0
v 170 - 460	= 29 sets of $\sum u, \sum v, \sum u^2 \sum v, \sum uv \alpha \beta \rho$ for the various ranges

Table 9. The allocation of addresses in the computer store.

The flow diagram (appendix 7) gives an outline of the process. It is seen that, after reading in the data, the computer completed the first iteration rapidly by using the pre-calculated information. However, if further iterations were necessary the full method was used, since it was not known what the new value of b would be. The number of iterations was limited to a maximum of four to save time. A greater number than this suggested an error, since the results should normally be achieved after two. When a satisfactory set of constants had been evaluated the results were printed. In the case of the 19 point range the curve fitting errors were printed against the appropriate load. The other ranges were then calculated by the above method once the limits of the range had been selected (table 10).

The first series of ranges was from 150 to 450 grams, the upper limit increasing by 50 grams for each range until the total number of ranges was six. The next series was now started at 200 grams and reached to 500, the upper limit again being increased by 50 gram intervals for each range until the total number of ranges was six. Three more series starting at 250, 300 and 350 grams were calculated, each having six ranges producing a total number of ranges of 30. The range limits and the constants for

```
+528
 +10 +100  +19  -0.037  +0.537  +0.510 +3
+10  +5
+15  -1
+19  -4
+24  -1
+29  -5
+34  -3
+39  +0
+44  +3
+49  +3
+54  +4
+59  +0
+64  +0
+69  +2
+74  -2
+79  +1
+84  +2
+89  -1
+95  +2
+100  -5
 +15  +44   +7  -0.049  +0.480  +0.440 +2
 +15  +49   +8  -0.047  +0.487  +0.451 +2
 +15  +54   +9  -0.045  +0.495  +0.462 +2
 +15  +59  +10  -0.039  +0.517  +0.490 +2
 +15  +64  +11  -0.037  +0.529  +0.505 +2
 +19  +44   +6  -0.053  +0.471  +0.424 +2
 +19  +49   +7  -0.048  +0.485  +0.448 +2
 +19  +54   +8  -0.044  +0.498  +0.467 +2
 +19  +59   +9  -0.036  +0.529  +0.509 +2
 +19  +64  +10  -0.032  +0.544  +0.528 +2
 +19  +69  +11  -0.032  +0.545  +0.529 +2
 +24  +49   +6  -0.060  +0.465  +0.403 +2
 +24  +54   +7  -0.047  +0.491  +0.453 +2
 +24  +59   +8  -0.032  +0.541  +0.527 +2
 +24  +64   +9  -0.028  +0.561  +0.552 +2
 +24  +69  +10  -0.029  +0.557  +0.547 +2
 +24  +74  +11  -0.026  +0.569  +0.562 +2
 +29  +54   +6  -0.022  +0.572  +0.578 +2
 +29  +59   +7  -0.012  +0.642  +0.649 +2
 +29  +64   +8  -0.012  +0.640  +0.648 +3
 +29  +69   +9  -0.017  +0.605  +0.612 +3
 +29  +74  +10  -0.016  +0.611  +0.619 +3
 +29  +79  +11  -0.020  +0.587  +0.592 +3
 +34  +59   +6  -0.004  +0.711  +0.714 +3
 +34  +64   +7  -0.007  +0.678  +0.687 +3
 +34  +69   +8  -0.016  +0.610  +0.619 +3
 +34  +74   +9  -0.015  +0.617  +0.627 +3
 +34  +79  +10  -0.021  +0.584  +0.586 +3
 +34  +84  +11  -0.026  +0.563  +0.558 +3
+4
```

Table 10. Complete computer output for one set of results.

each of these were printed. On completion of the 30 ranges the data for the next specimen were read into the computer and the process repeated.

A typical print-out for a specimen is given in Table 10. The number at the top gives the specimen number, the first two values on the next line are the limits of the range (corrected for machine calibration), followed by a number giving the total number of values in the range. The three constants c, k and b respectively are given next, followed by the number of iterations required. The next 19 lines give the loads and their corresponding error values which have been multiplied by 10,000 for ease of reading. The remaining 30 lines give the details of the various ranges in a similar fashion to the first line (note all loads are divided by 10).

F. COLLAGEN CONTENT OF SKIN

The collagen content of specimens was expressed as a percentage of the dry fat-free weight of the skin sample. The wet weight of the sample was obtained, but in the crushing process following freezing with liquid nitrogen there was a small loss of material which introduced inaccuracies into any expression of collagen content as a percentage of wet weight. The results are given in figures 49 - 52 in which the values for skin from male and female forearms and abdomens are expressed with respect to age. It was found that for skin from male forearms and abdomens and female arms, the percentage of collagen did not alter with age. Male abdominal skin contained 70.1% collagen (standard deviation $\pm$ 4.4) over the whole age range of 0 to 90 years. It was found that the values of the collagen content of both male and female forearms was very similar - mean 60.5% (standard deviation $\pm$ 3.6) and 61.7% (standard deviation $\pm$ 2.54) respectively.

There was a suggestion in female abdominal skin of some variation with age. The mean value in the first five years was 65%. At the ages of 24 and 53 years values of 75% collagen content were found, and in the ninth decade a mean collagen content of 67% was found. The data are too few to draw any statistical conclusions.

FIG. 49
VARIATION OF COLLAGEN CONTENT WITH AGE
FEMALE ABDOMEN

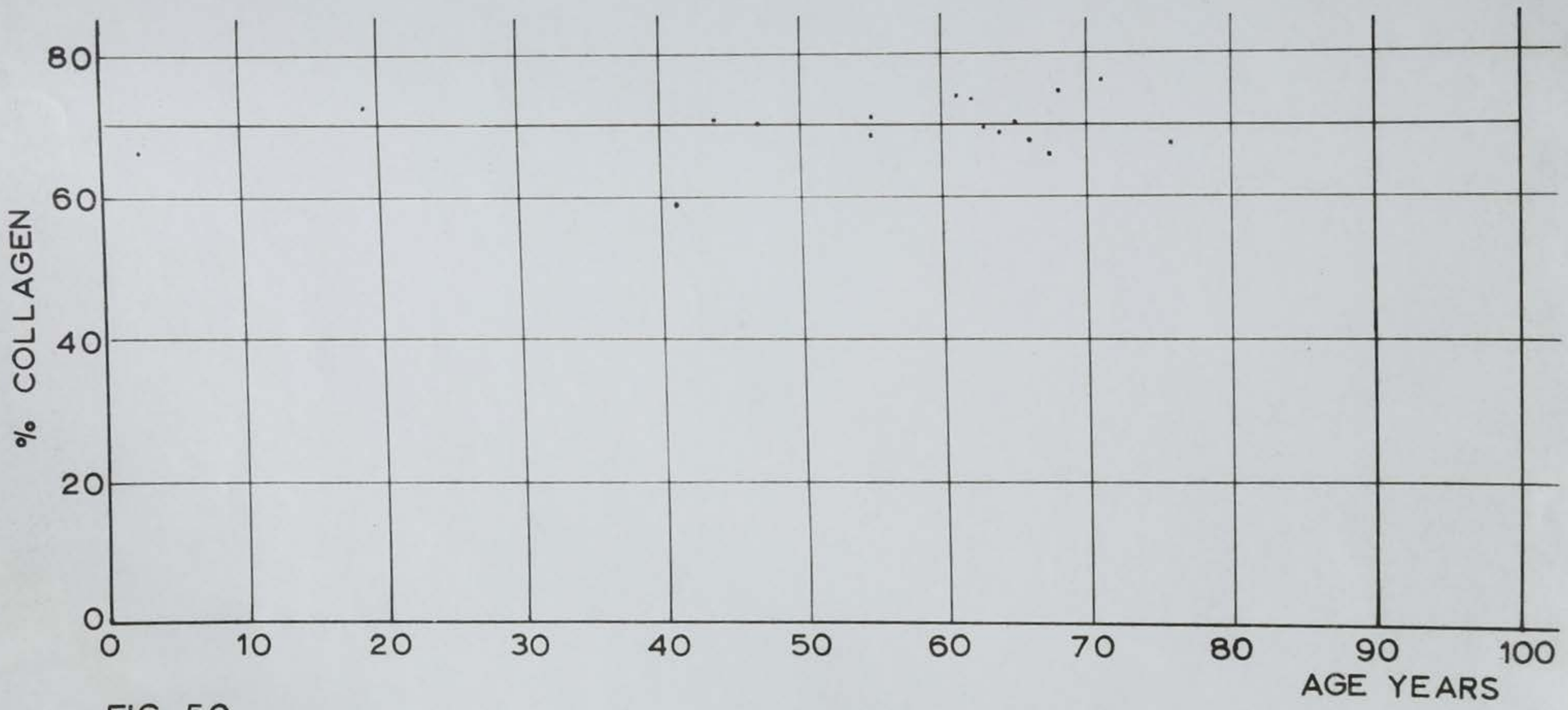


FIG. 50

VARIATION OF COLLAGEN CONTENT WITH AGE
MALE ABDOMEN

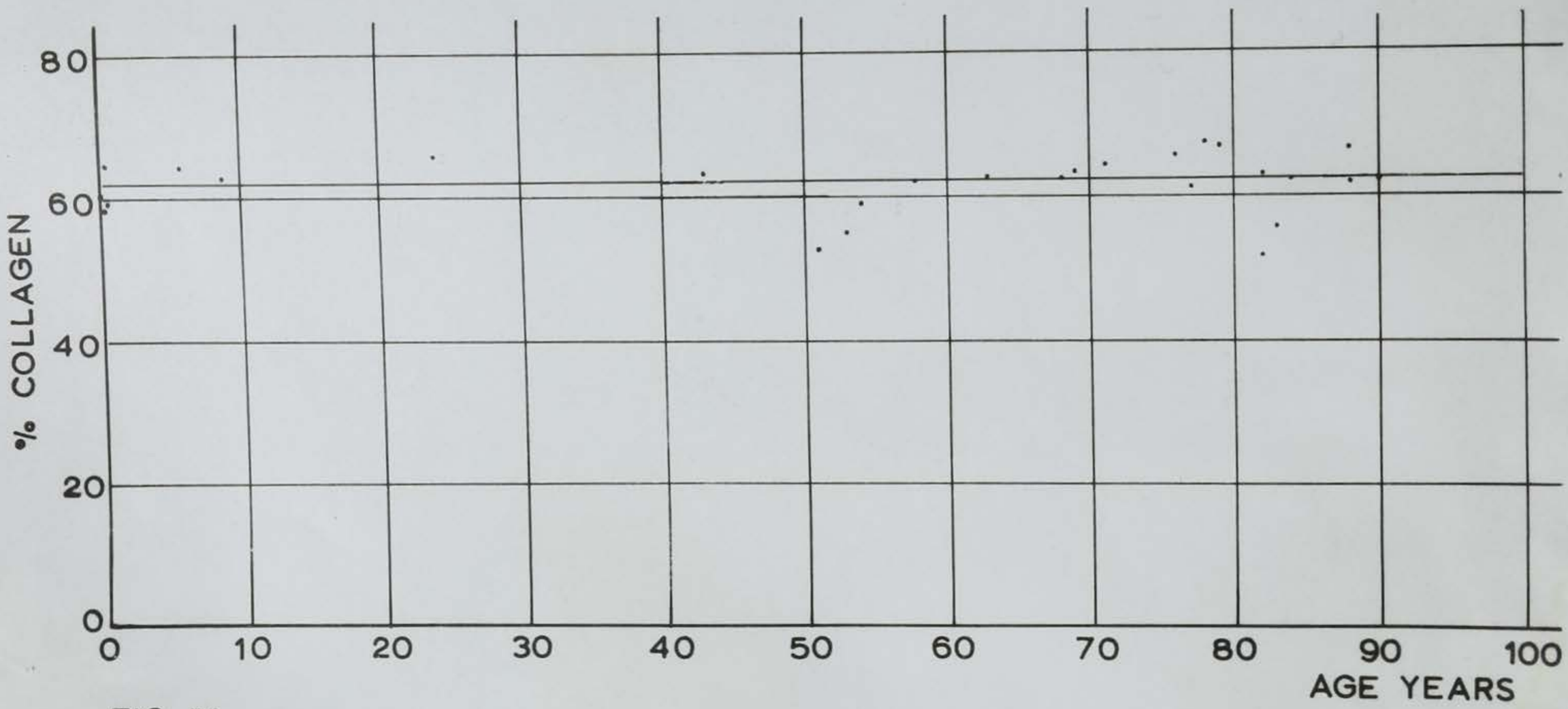


FIG. 51

VARIATION OF COLLAGEN CONTENT WITH AGE
FEMALE FOREARM

FIG. 52

VARIATION OF COLLAGEN CONTENT WITH AGE
MALE FOREARM

Values in pathological conditions.

The comparison of collagen content of normal skin with that obtained in various pathological conditions is given in Tables 11 and 12.

Subjects	% collagen
Non-arthritic (mean)	60.5
Rheumatoid arthritis (case 1)	58.3
Felty's syndrome (case 2)	56
Rheumatoid arthritis (case 3)	51.8
Osteoporosis (case 4)	63

Table 11. Comparison of collagen content in the skin from forearms of men without arthritis, three patients with rheumatoid arthritis and one with osteoporosis.

Subjects	% collagen
Non-arthritic	70.1
Felty's syndrome (case 2)	68
Rheumatoid arthritis (case 3)	67.9

Table 12. Comparison of collagen content in the skin from abdomens of men without arthritis and two patients with rheumatoid arthritis.

The details of the cases quoted in the above tables are given in paragraph G2 of this section. The patients

with rheumatoid arthritis had a collagen content somewhat lower than the mean value of skin from the same site in non-arthritic subjects of the same sex. There was no significant difference in the collagen content of skin from a patient with osteoporosis following pregnancy compared with that of other subjects.

G. EFFECTS OF PHYSIOLOGICAL AND PATHOLOGICAL CHANGES

1. PHYSIOLOGICAL CHANGES

For ease of presentation the values of the rheological constants have been shown in a series of graphs (figures 53 - 64) in which the constants have been related to age for each site and sex.

Age.

The constants b, k and l were found to vary with age. The relation of the constant b to age in abdominal skin is shown in figures 53 and 54. The males varied in age from 3 days to 87 years; the age range in females was 60 days to 90 years. It will be seen from the form of the b/age curves that there was a peak in the region of 40 years for both sexes. The curve was asymmetrical; the lowest values were recorded in the ninth decade. In skin from female forearms a similar curve was produced (figure 55), although the differences were not so striking. It will be seen that the scatter for skin from male forearms was considerable and no correlation with age could be deduced (figure 56).

Due to the relative scarcity of specimens in the second, third and fourth decades, data was scanty in this region. There can be no doubt, however, about the significance of the difference in the value of b in the

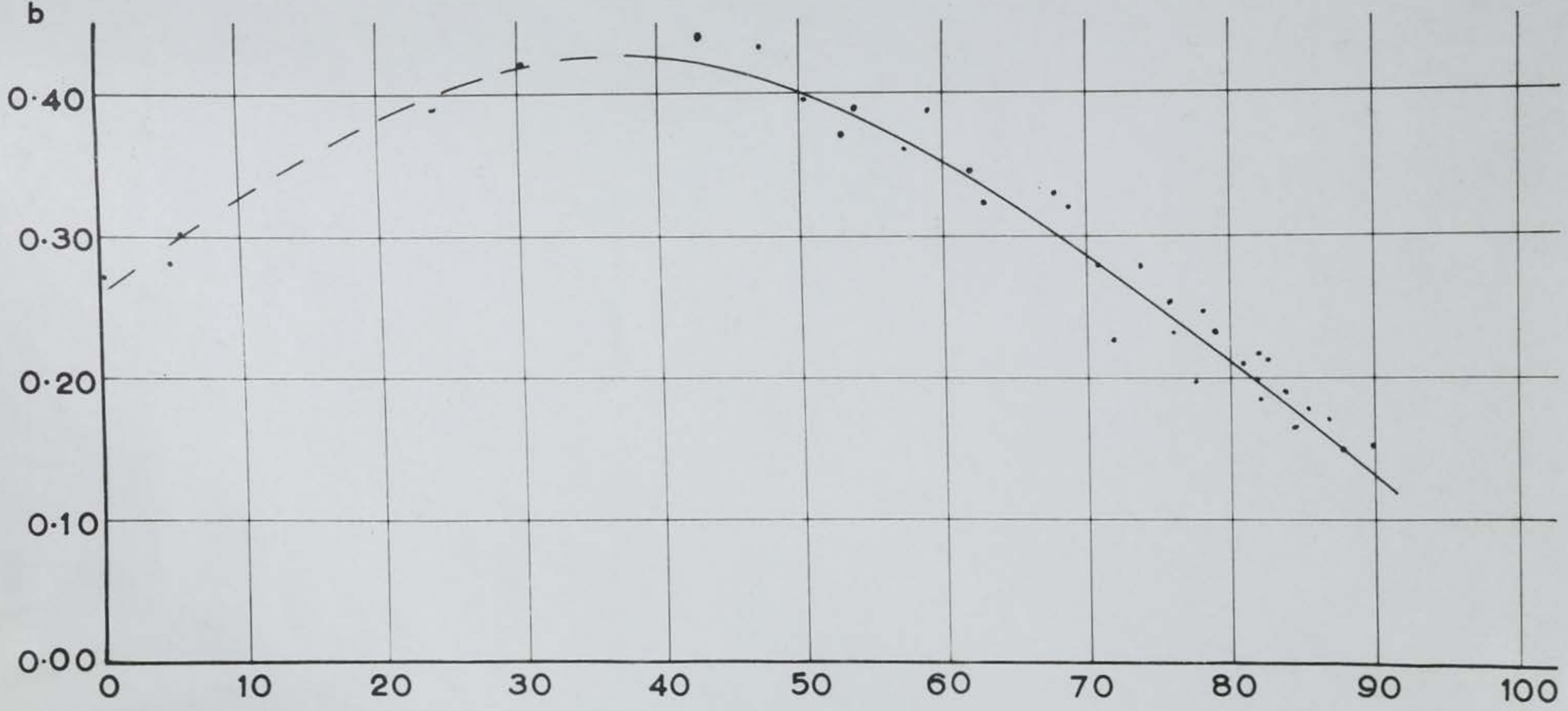


FIG. 53

VARIATION OF b WITH AGE
FEMALE ABDOMEN

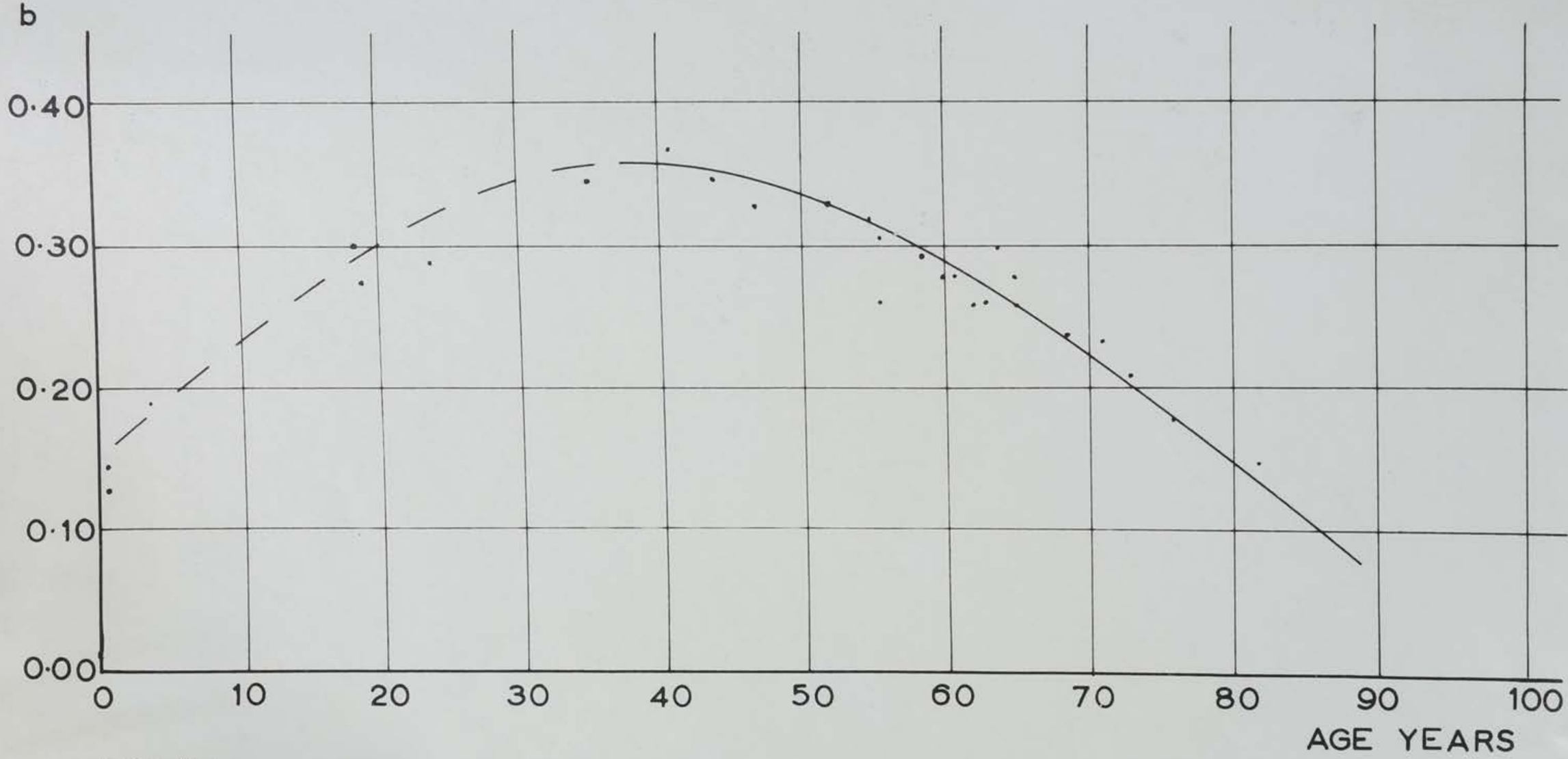


FIG. 54

VARIATION OF b WITH AGE
MALE ABDOMEN

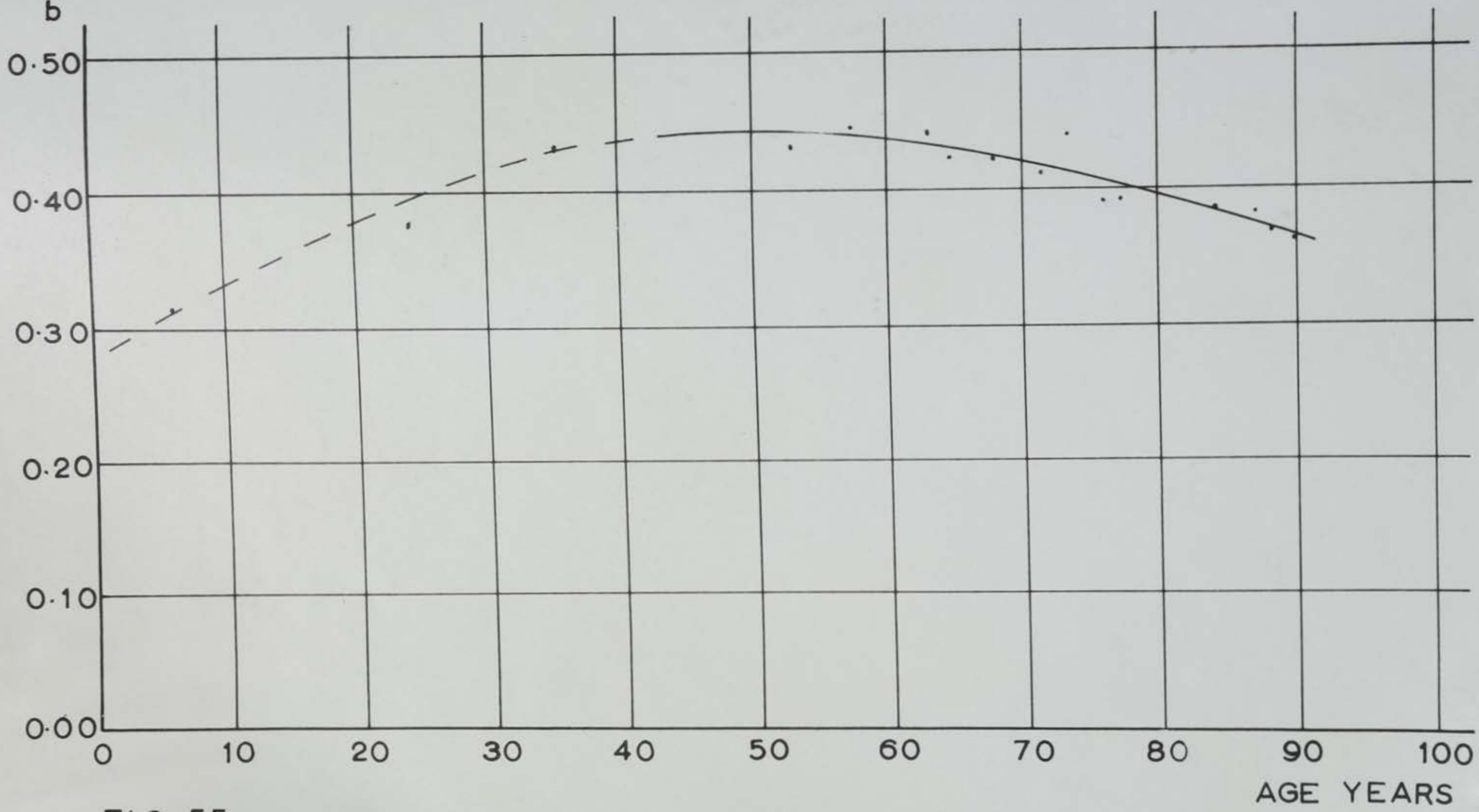


FIG. 55

VARIATION OF b WITH AGE
FEMALE ARM

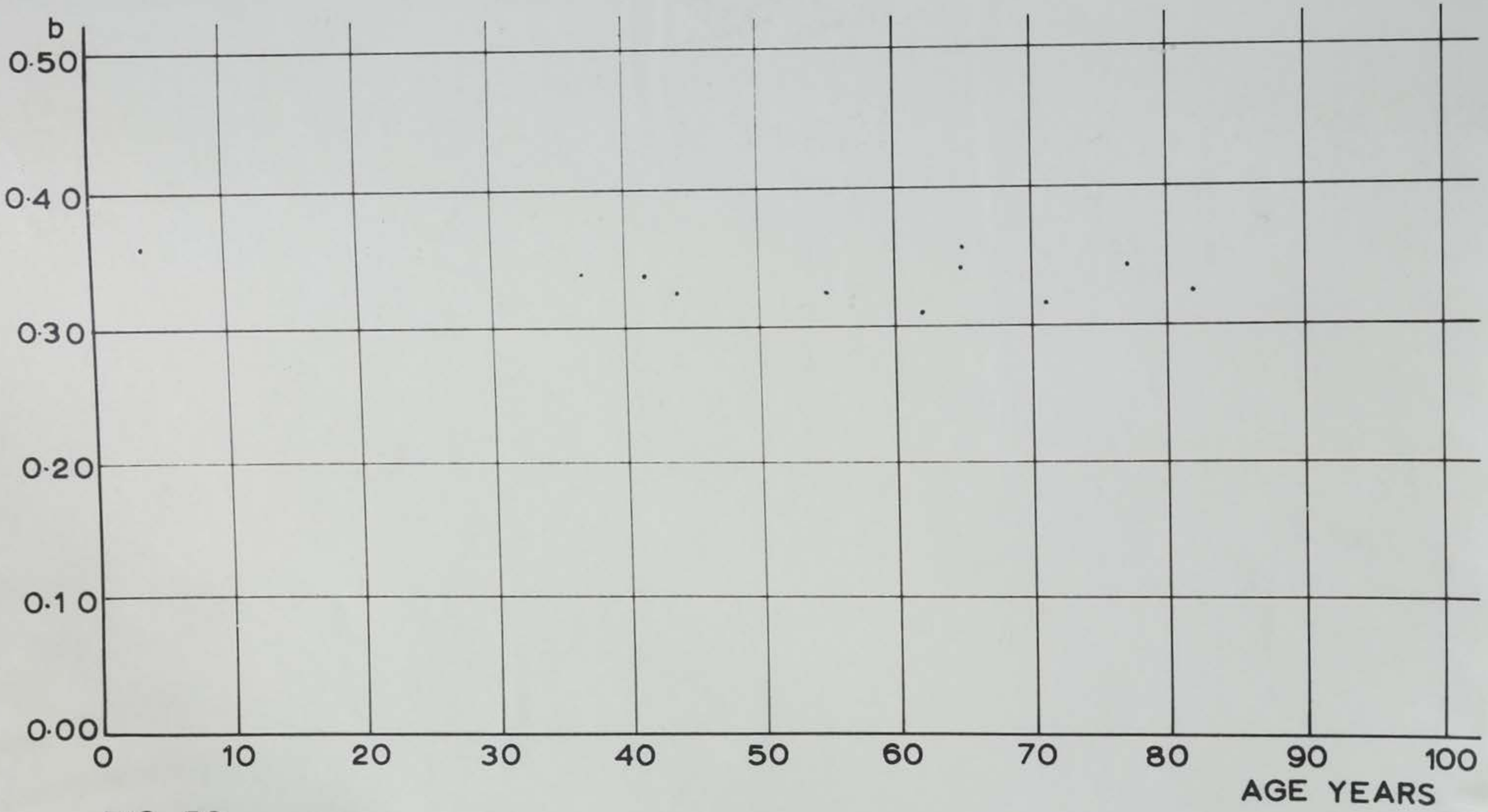


FIG. 56

VARIATION OF **b** WITH AGE
MALE FOREARM

decade 55 - 64 (mean 0.35 s.d. ± 0.016) compared with the decade 75 - 84 (mean 0.22 s.d. ± 0.009) for female abdominal skin. For male abdominal skin for the same decades, a similar drop in the value of b was observed. From the age of 40 years and over there was a striking correlation between age and the constant b for the skin from male abdomens ($r = 0.985$, $P < 0.001$), female abdomens ($r = 0.982$, $P < 0.001$) and female forearms ($r = 0.972$, $P < 0.001$). This correlation was not seen in male forearms ($r = 0.05$, $P > 0.1$).

The constant k became less with advancing age in abdominal skin (figures 57 and 58). There was a good statistical correlation between the value of the constant and age for male abdominal skin ($r = 0.886$, $P < 0.001$) and for female abdominal skin ($r = 0.824$, $P < 0.001$). There were no correlations between the constant k and age for the forearm skin of females ($r = 0.35$, $P > 0.1$) or males ($r = 0.17$, $P > 0.1$).

The constant l followed a similar pattern of reduction with advancing age in abdominal skin (figures 61 and 62). There was a significant correlation statistically in males and females, $r = 0.946$, $P < 0.001$ and $r = 0.895$, $P < 0.001$ respectively). In the skin of female

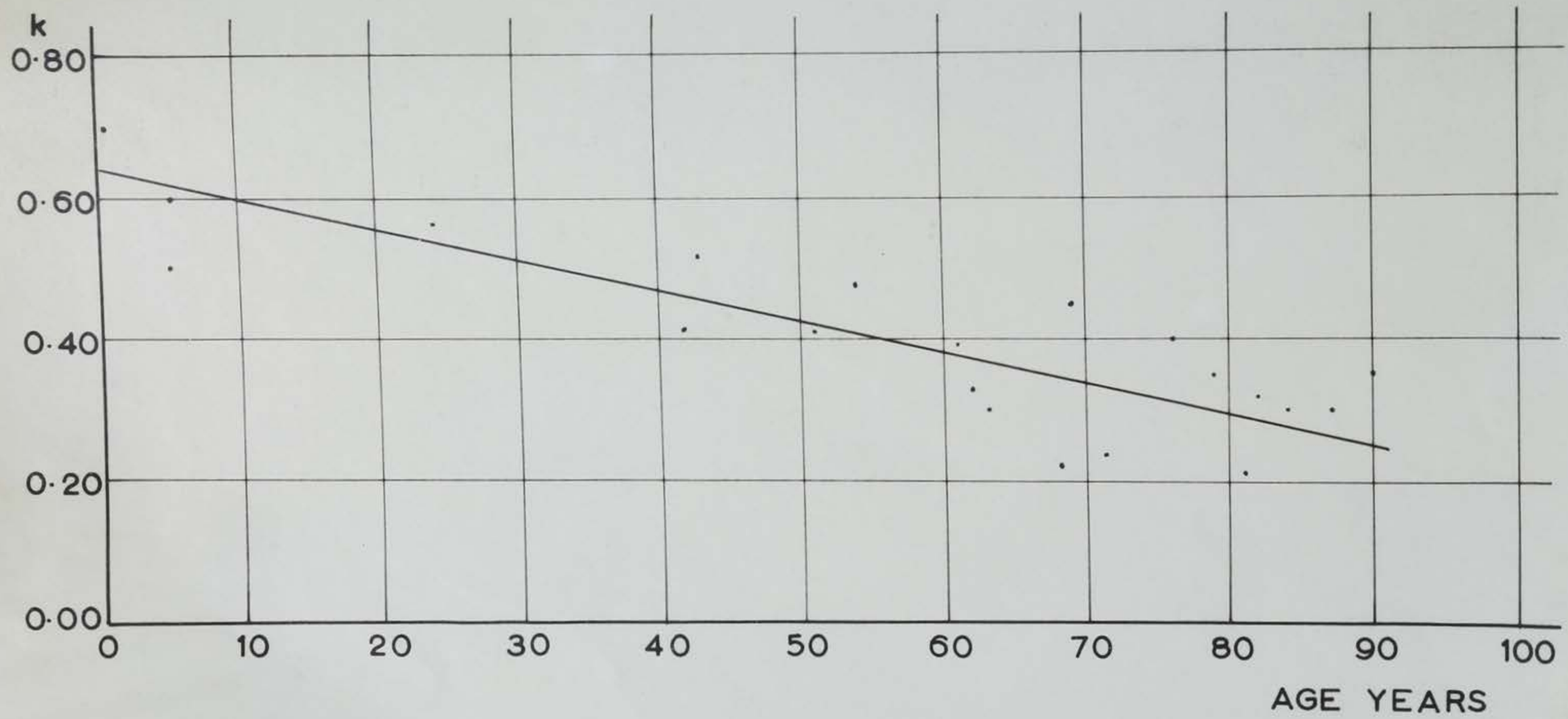


FIG. 57

VARIATION OF k WITH AGE
FEMALE ABDOMEN

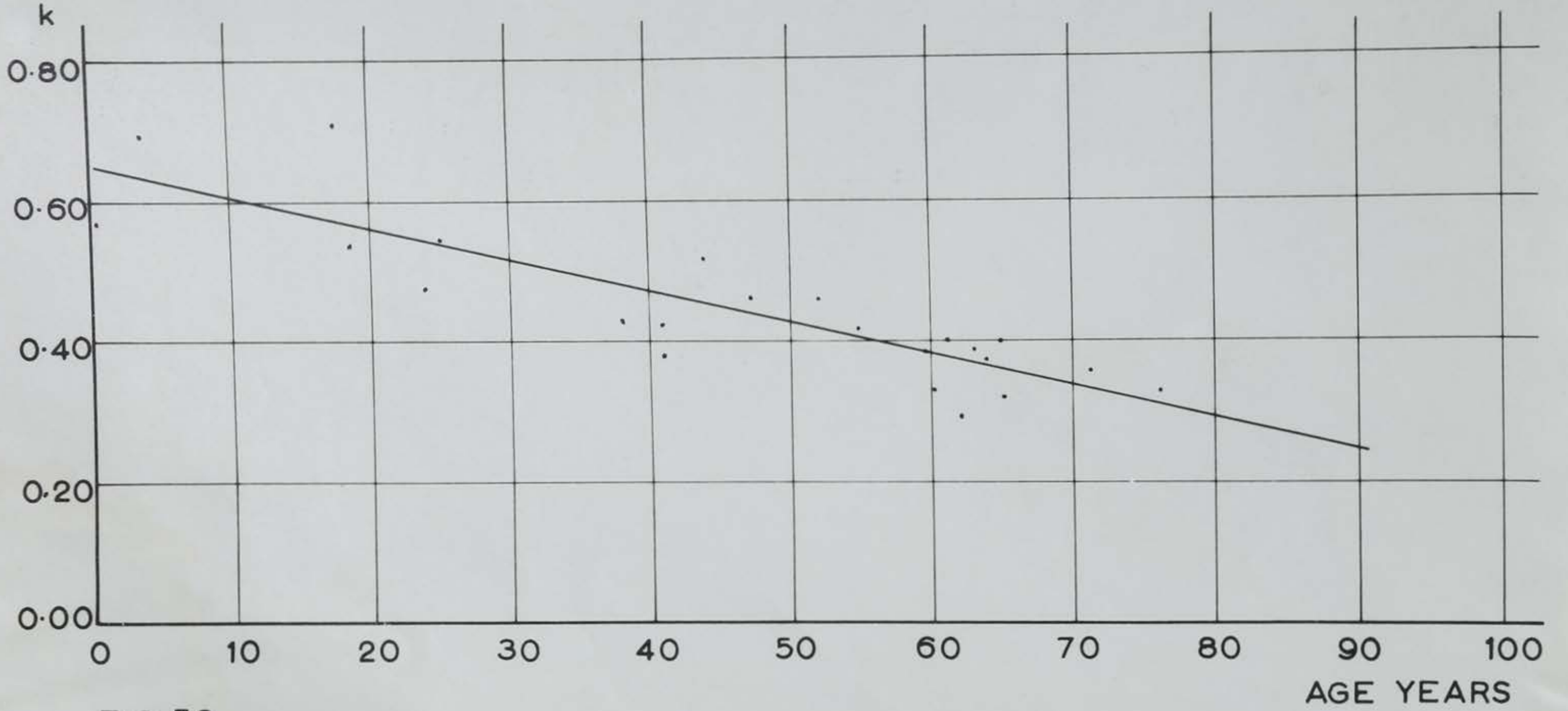


FIG. 58

VARIATION OF k WITH AGE
MALE ABDOMEN

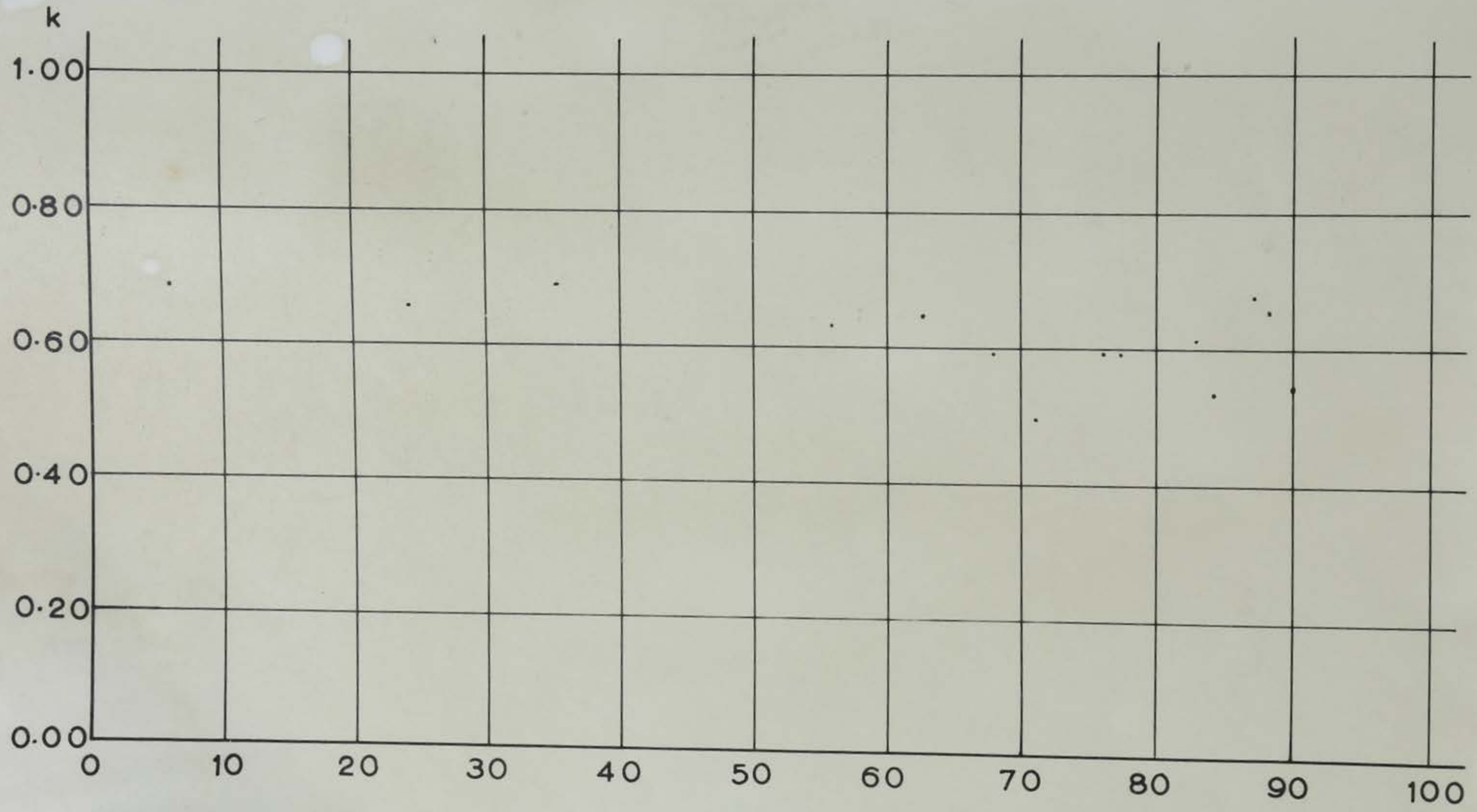


FIG. 59

VARIATION OF k WITH AGE

FEMALE ARM

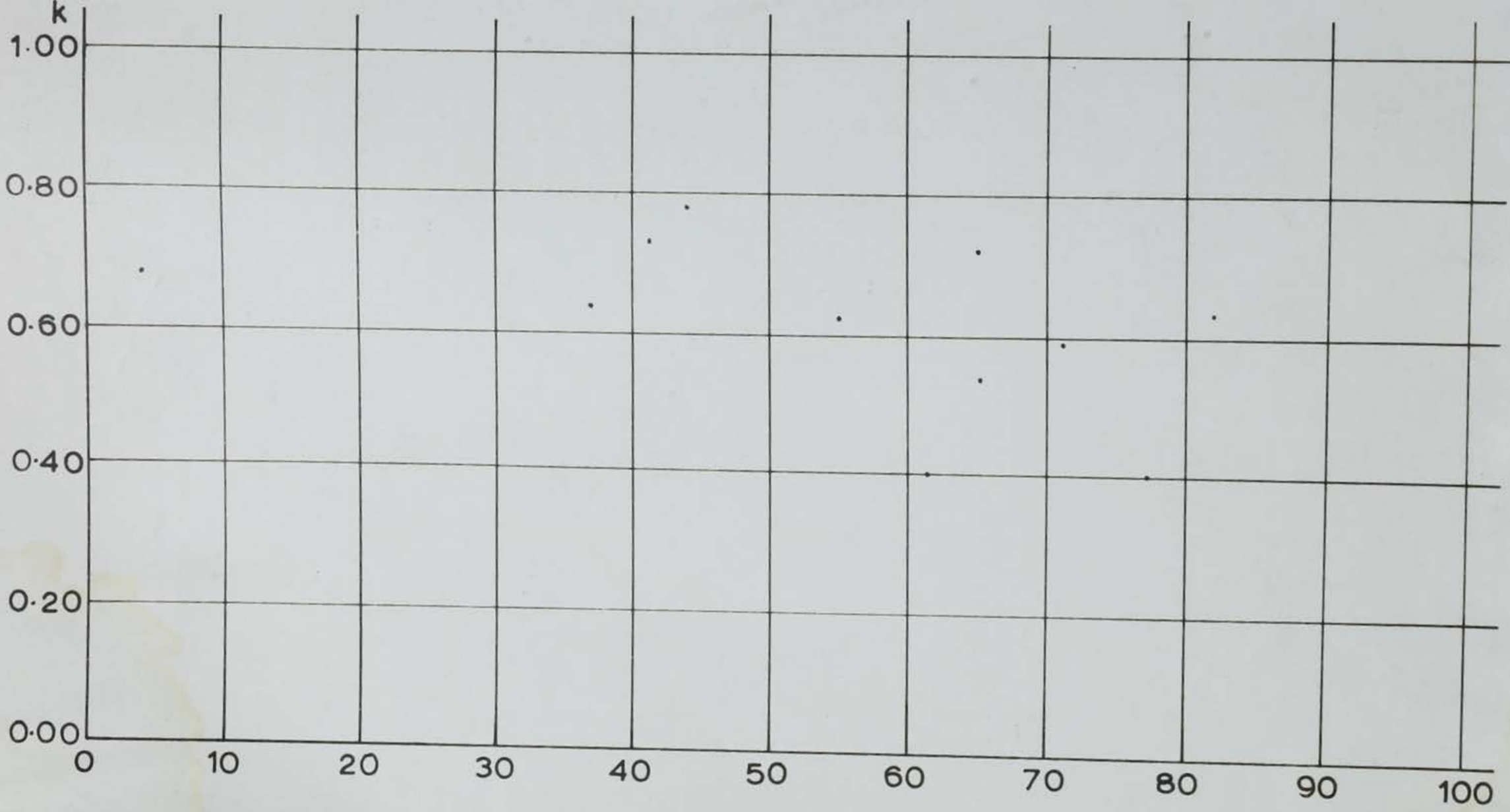


FIG. 60
VARIATION OF k WITH AGE
MALE FOREARM

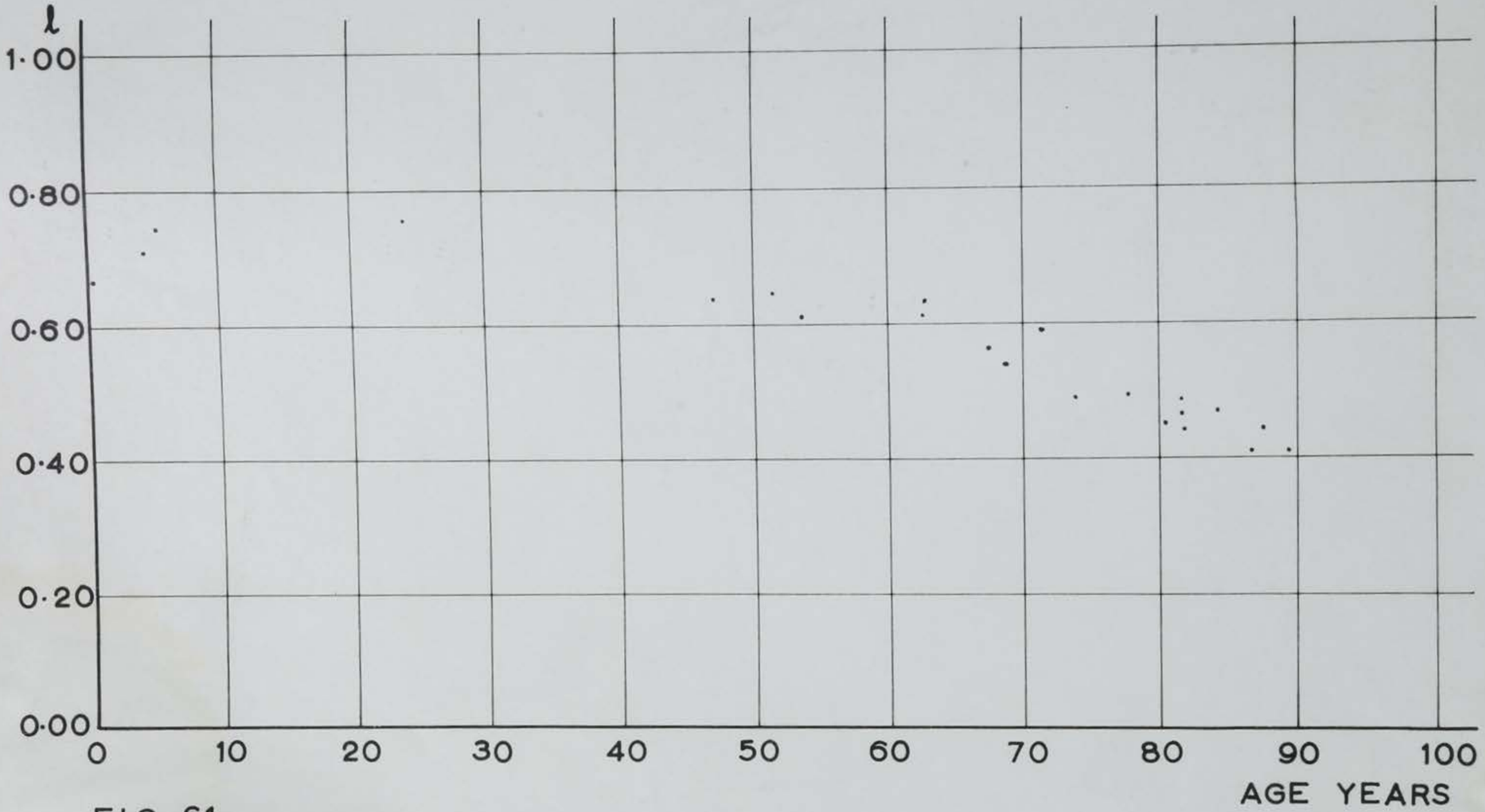


FIG. 61

VARIATION OF l WITH AGE
FEMALE ABDOMEN

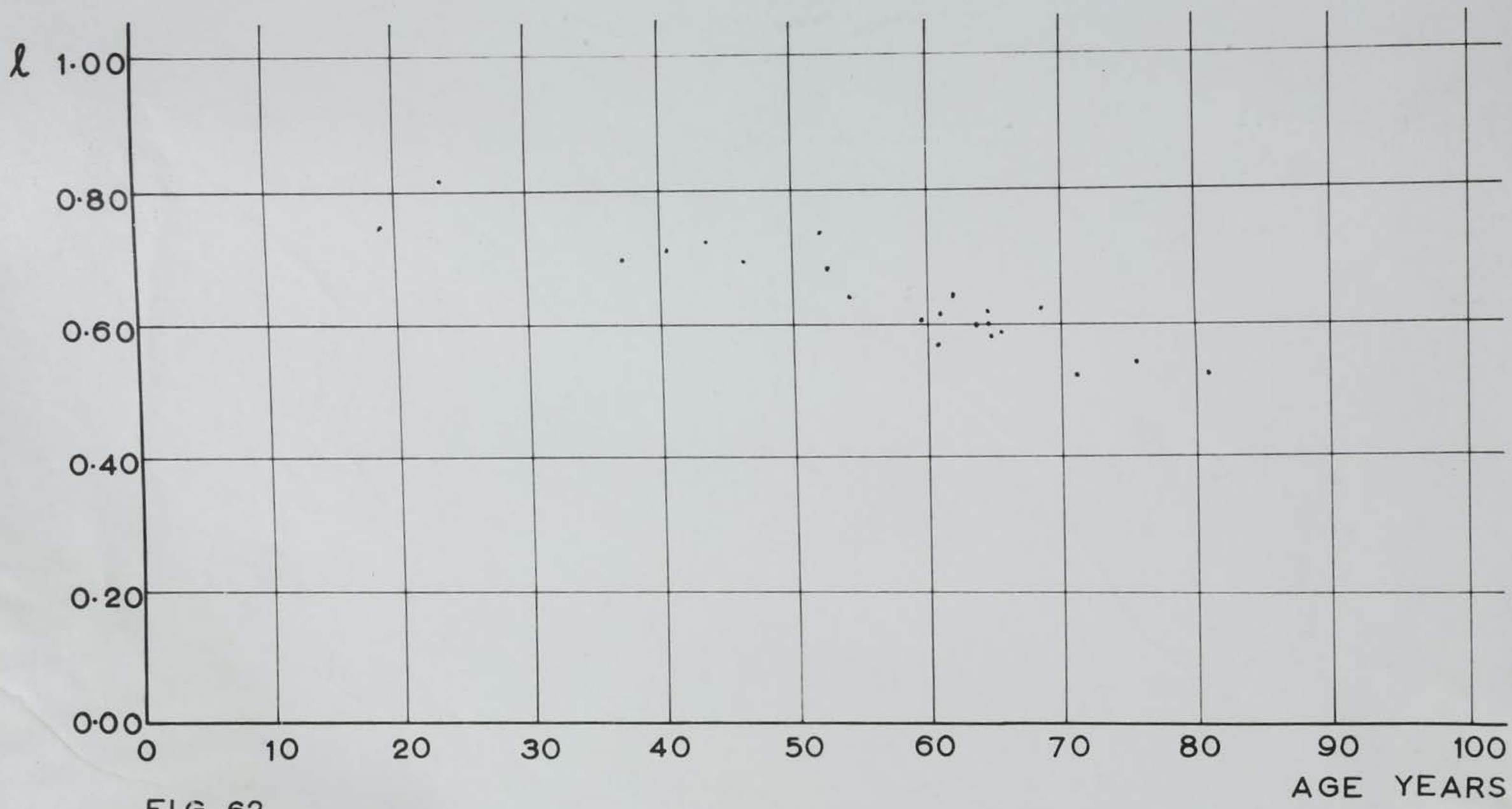


FIG. 62

VARIATION OF l WITH AGE
MALE ABDOMEN

forearms there was a similar reduction with age (figure 63) which was statistically significant ($r = -0.975$, $P < 0.001$). The relation in the forearm skin of males was not significant ($r = -0.50$, $P > 0.1$).

Sex.

In both sexes the relationship of the constants to age followed a similar pattern. With abdominal skin the value of the constant b was about 0.07 higher throughout the complete age range (figures 53 and 54).

Throughout the age range the constant k was similar for both sexes and the constant l was also similar in the two sexes (figures 57 - 64). There was no significant difference between them.

Site.

Comparison of the difference in the constants according to site was investigated for specimens from the abdomen, forearm, back and thigh. Tables 13 and 14 show the results.

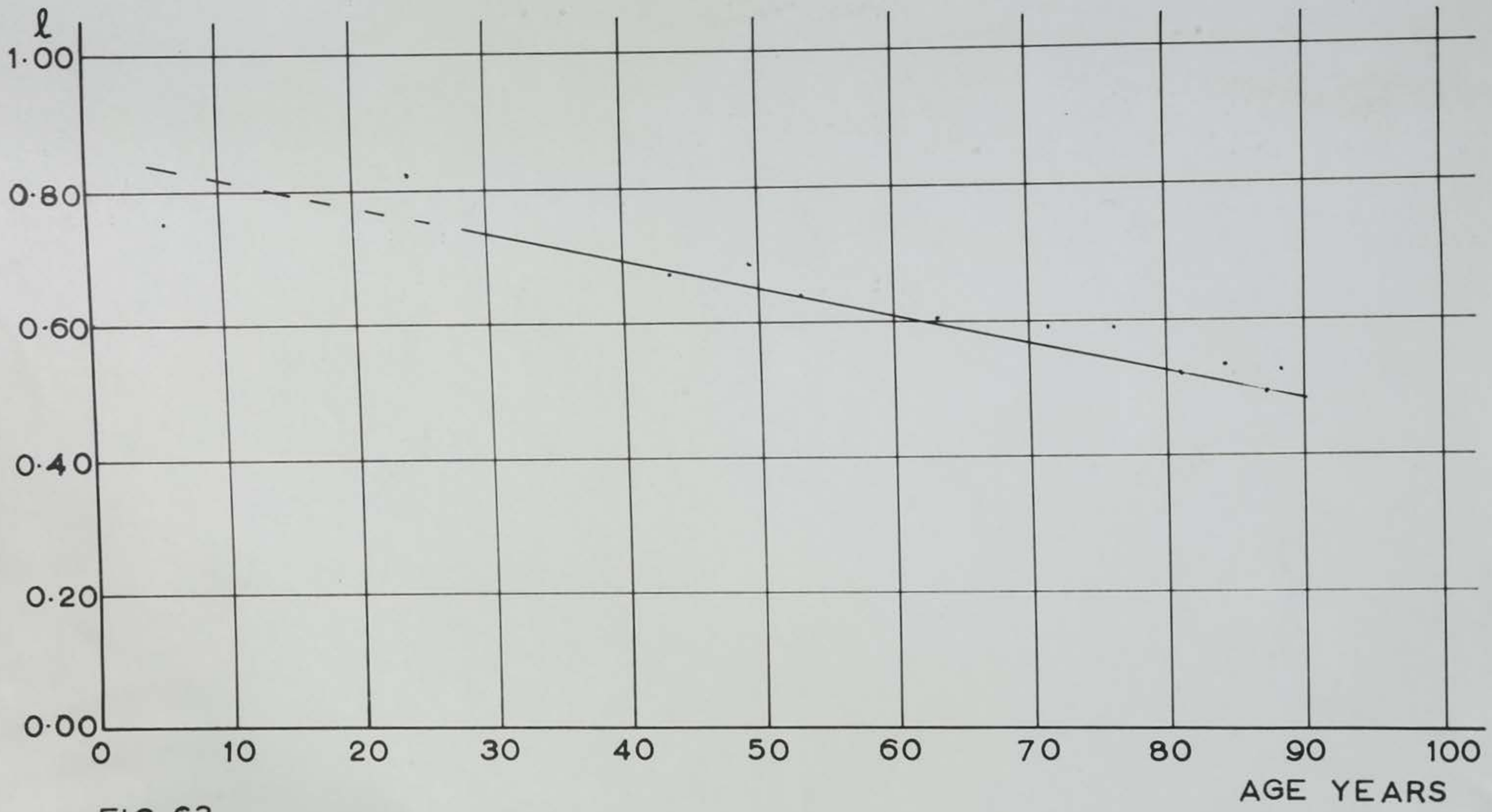


FIG. 63

VARIATION OF l WITH AGE
FEMALE ARM

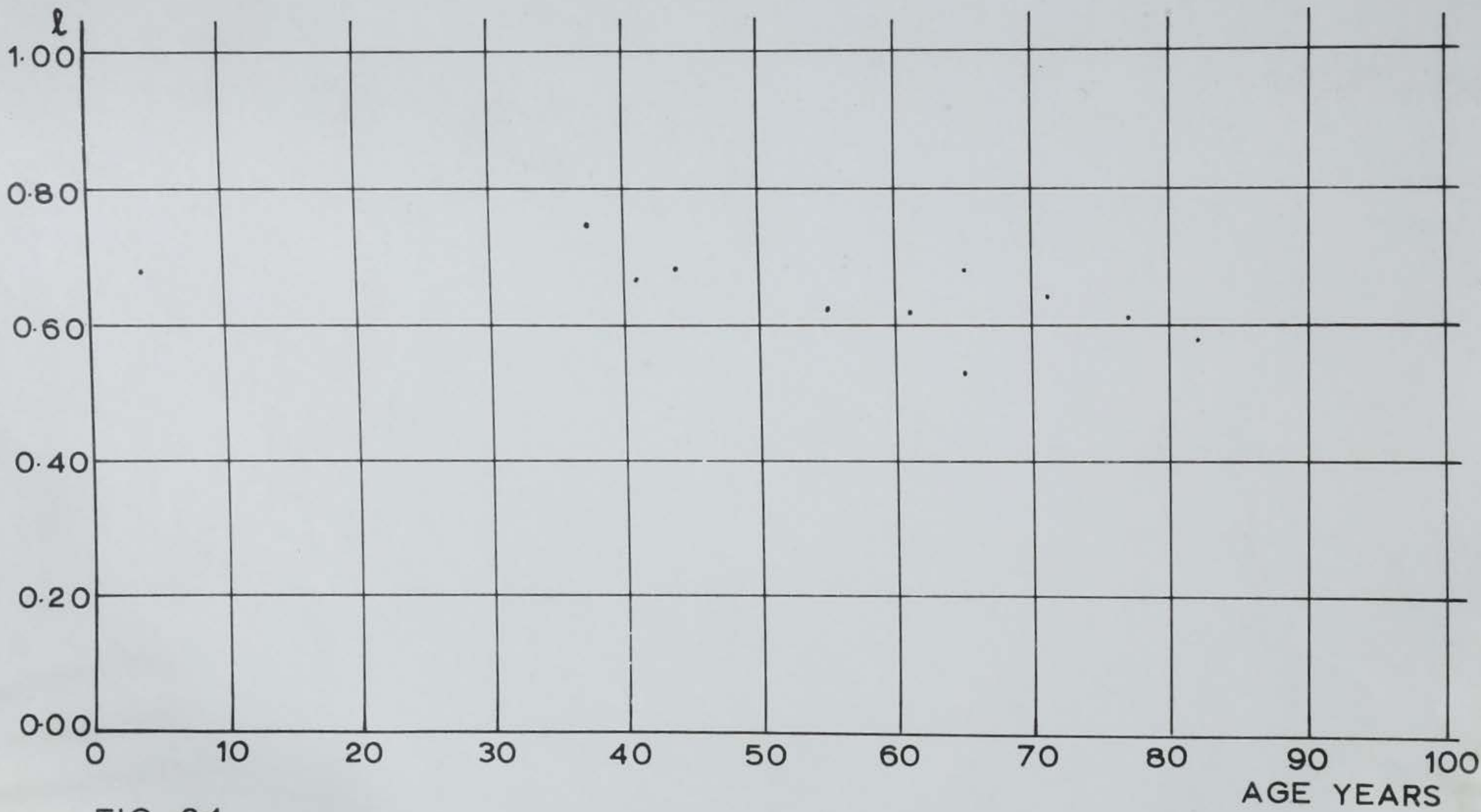


FIG. 64

VARIATION OF ℓ WITH AGE
MALE FOREARM

Site	l	k	b
abdomen	0.44	0.21	0.20
back	0.50	0.25	0.15
forearm	0.53	0.46	0.37

Table 13. Comparison of rheological constants for skin from the abdomen, back and forearm in a woman aged 81 years.

Site	l	k	b
abdomen	0.75	0.56	0.38
forearm	0.81	0.65	0.37
thigh	0.75	0.53	0.19

Table 14. Comparison of rheological constants for skin from the abdomen, forearm and thigh in a 24 year old woman.

Most specimens were obtained from the abdomen and forearm. The comparison of the constant b in relation to age for the skin of female abdomens and forearms is shown in figures 53 and 55. Both graphs were of similar form but in the latter decades there was much less reduction in the value of b with advancing age in forearm skin than there was in abdominal skin. The fall in this part of the curve for forearm skin was from 0.44 to 0.36 compared with a fall of 0.44 to 0.14 for abdominal skin.

In the earlier decades the increase in value was similar (0.27 to 0.44 from birth to 40 years of age) in both sites.

The values of the constant k for the skin from female abdomens and forearms are shown in figures 57 and 59. Over the complete age range the reduction was less for forearm skin than abdominal skin. In the seventh and eight decades the values of k appeared higher for the skin of the forearms than for that of the abdomen, but this difference is not significant.

The values of the constant l for the skin from female abdomens and forearms are shown in figures 61 and 63. There was no significant difference between them over the age range.

2. PATHOLOGICAL CHANGES

Rheumatoid arthritis.

Case 1.

A man aged 59 had early active rheumatoid arthritis of 5 months duration. There was severe restriction of shoulder movements, mild diffuse swelling with severe limitation of movement of the fingers and thickening of the palmar fascia of the left hand. Behind both olecranon processes there were bursae within which were nodules. He was being treated with soluble aspirin. Investigations showed haemoglobin 68%, blood sedimentation rate 124mm. 1st. hour, differential agglutination test for rheumatoid arthritis positive in a titre of 64. There were erosive changes in the x-rays of the shoulders and hands, typical of rheumatoid arthritis.

A biopsy of forearm skin was taken. The rheological constants are shown in Table 15.

	l	k	b
non-arthritic	~ 0.60	~ 0.50	0.32
rheumatoid	0.54	0.39	0.27

Table 15. Comparison of rheological constants for forearm skin in a man with rheumatoid arthritis compared with a man of the same age without arthritis.

Felty's syndrome.

Case 2.

A man aged 66 had Felty's syndrome (i.e. rheumatoid arthritis, splenomegaly and leucopaenia). He had widespread rheumatoid arthritis for nine years which had been treated with prednisolone 5mg. b.d. Seven years after the onset of the arthritis he was admitted to St. James Hospital, Leeds, for investigation of leucopaenia (white count 2,200). At that time haemoglobin was 67%, blood sedimentation rate 116mm. in the first hour. He was re-admitted to the General Infirmary at Leeds 18 months later gravely ill with a haemoglobin of 39%, a profound leucopaenia (white count 250), a thrombocytopaenia and a high blood sedimentation rate. After suitable preparation a splenectomy was done and a moderately large spleen (15 cms. in its long axis) was removed. There was excess iron accumulation, and alternation in the connective tissue of the arteries and of the fibrous trabeculae. The arterial changes were of prominent hyaline walls. The pathologist commented that these were rather similar to the lesion seen in systemic lupus.

Post-operatively he did very well. His haemoglobin was eventually 66%, the white count was 4,100 though a

neutropenia persisted. The platelets returned to normal.

His arthritis settled down extremely well, and he was discharged on enteric coated prednisolone 2.5mg. q.d.s. He was re-admitted 7 months later, with a three week history of cough, dysphagia and left sided chest pain exacerbated by inspiration. Therapy during this admission comprised steroids, antibiotics and transfusion. A coagulase positive staph. aureus was isolated from the peripheral blood fifteen days after admission. A pulmonary infarct was evident radiologically and multiple pyaemic dermal abscesses developed. Death occurred 36 days after admission.

At autopsy the body was that of a thin, elderly man, the trunk and arms showed multiple petechial haemorrhages and several small areas of pressure necrosis were present on the buttocks and sacrum. On opening the chest the left lingular lobe was found to be adherent to the parietal pleura. There were no effusions. Skin was taken for rheological examination and the results are shown in Table 16.

	l	k	b
Abdomen			
non-arthritic	0.60	0.35	0.25
Felty's syndrome	0.55	0.49	0.18
Forearm			
non-arthritic	~0.60	~ 0.55	0.35
Felty's syndrome	0.52	0.54	0.22

Table 16. Comparison of rheological constants for abdominal skin in a man with Felty's syndrome compared with a man of the same age without arthritis.

Rheumatoid arthritis.

Case 3.

A 60 year old man with longstanding rheumatoid arthritis of 25 years duration. The arthritis was severe in extent and destructive in character. Nearly every joint of the body was involved and four or five months prior to his last admission signs of spinal cord compression, due to cervical spine involvement, had developed. Three years previously a sterile empyema had been found in the pleural cavity. He was admitted to the Royal Bath Hospital, Harrogate, severely ill and died soon after. He had received prednisolone 5mg. b.d. for several years until the time of his death.

At autopsy the cause of death was found to be renal papillary necrosis. There was evidence of severe rheumatoid arthritis, an empyema on the left side of the chest and pleural adhesions bilaterally. Skin specimens were taken from the abdomen and forearm for study.

The collagen content is given in section V.F. On removal of a square inch of forearm skin it retracted along Langer's lines to 0.9 inch and there was no change at right angles to Langer's lines. The values of the rheological constants are summarised in Table 17.

	l	k	b
Forearm			
non-arthritic	0.60	0.50	0.33
rheumatoid	0.54	0.56	0.30
Abdomen			
non-arthritic	0.61	0.38	0.27
rheumatoid	0.52	0.68	0.20

Table 17. Comparison of rheological constants for forearm and abdominal skin in a man with rheumatoid arthritis compared with a man of the same age without arthritis.

It will be noted that the constant k is much greater in the rheumatoid patients. This is in keeping with the decreased collagen content found biochemically (see section V.F.). There is insufficient variation in the constant l to account for this difference, and the findings suggest that a variation in the constant n is responsible.

Case 4.

A 34 year old woman developed osteoporosis following pregnancy two years previously. The osteoporosis proved refractory to treatment.

A biopsy of forearm skin was taken and the rheological constants are summarised in Table 18. It will be seen that the constant k differs from a comparable control subject. The difference is such that it raises the possibility of a directional effect associated with Langer's lines, but there was no other evidence to suggest this.

	l	k	b
non-arthritic	0.69	0.72	0.42
osteoporotic	0.70	0.34	0.50

Table 18. Comparison of rheological constants for forearm skin in a woman with osteoporosis compared with a woman of the same age without osteoporosis.

Rheumatoid arthritis.

Case 5.

A man aged 54 had definite rheumatoid arthritis for 13 years. He had been on numerous medications including gold, butazolidin, prednisolone, triamcinolone, dexamethazone and betamethazone which he was receiving at the time of his final admission. He came in with a

coronary thrombosis and died soon after admission. Autopsy confirmed eccymoses of the hands and forearms, which had been noted before death. Examination of the knee joint showed cartilage to be lost over the articular surface and gross fibrosis. The capsule was whitish, fibrotic and shrunken. The pannus and fibrous union between articular surfaces could not be disected. The right ventricle was dilated and extensive white patches of sub-endocardial fibrosis overlaid the posterior wall. The septal papillary muscles of the mitral valve contained a khaki-coloured infarct. Patchy fibrosis involved the substance of the myocardium on the posterior wall of the left ventricle. There was severe atheromatous changes in the coronary arteries. The aorta showed moderate atheroma. There was severe oedema of both lungs and basal congestion. There was cortical scarring of the right kidney, suggesting old chronic pyelonephritis. The ribs were fragile and clearly osteoporotic.

Abdominal skin was taken for rheological studies and the values of the constants are shown in Table 19.

	l	k	b
non-arthritic	0.6	0.35	0.26
rheumatoid	0.59	0.47	0.2

Table 19. Comparison of rheological constants for abdominal skin in a man with rheumatoid arthritis compared with a man of the same age without arthritis.

Psoriatic arthritis.

Case 6.

A 25 year old man had psoriatic arthritis. He was admitted to the General Infirmary at Leeds for treatment of generalised erythrodermic psoriasis. Skin lesions had been present for six years and he had developed psoriatic arthritis two years previously. The arthritis had been moderate in extent, and was completely inactive on admission due to his high dose of steroids. Investigations showed haemoglobin 102%, blood sedimentation rate 2mm. 1st hour, white cell count 12,000/cm.mm. differential agglutination test negative (titre 2). X-rays showed mild erosive changes in the hands and feet, and moderate sacro-iliitis. He was receiving triamcinolone (an analogue of cortisone) in a dose of 8mg. four times daily. The skin had been treated with synalar (a steroid preparation for topical application). He had been on this regime ten days prior to biopsy.

A biopsy of skin was taken from the left forearm. The skin was erythrodermic due to involvement in the psoriatic process. It was noted to roll up spontaneously unlike any other specimens tested. On extension it yielded early and the stress strain curve was not suitable for rheological analysis.

Cases 7 - 9.

Biopsies were obtained from the forearms of a further three patients, but unfortunately the specimens yielded early during extension and these stress strain curves were unsuitable for rheological analysis. Case 7 was an example of osteoporosis in a 35 year old woman, who developed symptoms 3 or 4 months after pregnancy. Case 8 was a 52 year old man who was a miner with Caplan's syndrome. He had rheumatoid arthritis of two years duration and a radiological picture in the chest of nodular shadowing. He was not receiving steroids. Case 8 was a 35 year old man with rheumatoid arthritis of four years duration, which progressed rapidly so that he became very disabled. He was receiving prednisolone 2.5mg. t.d.s. at the time of admission.

PROTEIN DEPLETED RABBITS.

Two pairs of albino rabbits were selected at birth. One member of each pair was fed a normal diet while the other was fed a diet deficient in protein. On reaching maturity the rabbits were sacrificed and specimens of abdominal skin and aponeurosis taken. The skin was tested in the normal manner and the collagen content was determined. Mr. G. Tate tested the impact loading necessary to rupture sutured aponeurosis.

It was found that the thickness of the skin was somewhat less in the case of the depleted rabbits. Skin thickness of normally fed rabbits was 0.049 inch, and that of protein depleted rabbits was 0.045 inch. The collagen content dry weight was essentially the same in both - 80.0% for normal rabbits and 81.2% for protein depleted rabbits. The results from the extensometer indicated that once compensation had been made for the reduction in thickness, there was no significant difference in the values of the constants (Table 20).

	l	k	b
normal	0.95	0.37	0.38
depleted	0.88	0.40	0.40

Table 20. The rheological constants of the skin of normal rabbits compared with protein depleted animals.

VI. DISCUSSION

The science of rheology can probably be taken as dating from the time of Hooke (1660) who enunciated the classical law which bears his name. This law may be regarded as one of the foundations of the science, being first presented in the form "ut tensio sic vis" ("The extension of any spring is directly proportional to the force applied").

From the time of Hooke a great many workers (Marriotte, 1686; Bernoulli, 1705; Coulomb, 1776; Young, 1807; Navier, 1822; Euler, 1843) have contributed to the general theory of elasticity. Most materials however fall outside the scope of this theory because they fail to comply with Hooke's law.

Many workers (Wall, 1942a; Frenkel, 1946; Hall, 1951a) have tackled the difficult problem of producing theories to describe the properties of materials which behave in this more complex manner and which exhibit viscous or plastic characteristics. The term rheology is now used to describe the physical properties of materials whether they exhibit elastic, plastic or viscous phenomenon.

Numerous attempts have been made to investigate the

physical properties of biological tissues by rheological means. The value of much of this work has been reduced by the fact that many of the techniques were based on unsound theory (Dick,1949) and others set out to investigate a phenomenon which is not a simple property of the material. The Schade elastometer (Schade, 1912) is a typical example of the latter, where the indentation caused by a given point load is said to give information regarding the elasticity of skin in vivo. Unfortunately, other factors such as skin thickness, skin tension and the condition of subcutaneous tissue will mask the results.

An analytical description of joint stiffness in man and the cat has been given and a rheological model consisting of Maxwell, St. Venant and Kelvin elements connected in series synthesised to describe the behaviour (Johns and Wright, 1960, 1962 and 1964). These authors have also observed the effect of physiological and pathological factors on the rheological properties of the joint (Wright and Johns, 1960a and b, 1961).

By means of in vitro tests some workers have carried out valuable studies and investigated specific physical properties of the material (Bergel, 1961 a and b; Kenedi, 1964a). The increase of tensile strength of skin with age has been demonstrated by Rollhauser (1950)

working with specimens accurately measured. He also showed increased stiffness with age, which has been confirmed by the present work. Changes in the elastic properties of the aorta with age have been investigated by Krafka (1942). Valuable work has been carried out on the properties of the major components of skin, namely collagen and elastin (Wood, 1954; Hall, 1964).

Extensometer.

The apparatus developed to carry out the present work operated on the constant rate of extension principle. This is more versatile than the constant rate of loading system used commonly in the biological field (Krafka, 1937; Hall, 1951 a and b; Morgan, 1960). The system used was one which has been adapted for many industries including engineering, leather and textiles. Its main advantages lie in the fact that tests may be carried out under widely differing conditions and the results presented in a convenient and accurate form.

As most of the commercial extensometers were large and cumbersome (e.g. the Instron) an instrument was devised specifically for testing skin. The specimens could be extended at any one of a number of rates and the results presented in the form of a load/extension curve. This was an advantage over many systems, in which it was necessary to take readings manually. Manual reading limits accuracy and curtails the number of readings

possible at higher rates of extension (Green and Loughborough, 1945; Conabere and Hall, 1946). Various other features were included in the extensometer, so that investigation of stress relaxation, tensile strength and permanent set could be made. Provision was also made for testing the specimen under a temperature controlled liquid, a feature not always included on other instruments (Conabere and Hall, 1946; Roddy, 1952).

When conducting experiments where comparison is to be made between various specimens it is necessary to ensure that specimens are produced of identical size. In the present work the problem was overcome by means of cutting the specimen down to a given thickness on the microtome, when mounted either in a wax box or on a large asbestos slab. Rigidity of the sample for the purpose of cutting to obtain comparable specimens was achieved by freezing, since soluble waxes which solidified at room temperature (Annotation, 1960) proved unsatisfactory. The width and length were controlled by the use of a die. Other workers such as Harkness and Harkness (1961) have used a different system and have expressed their results in terms of load per unit of collagen. The total collagen has been determined by biochemical means in these

cases, and it must be assumed that the degree of fibre orientation remained unchanged for all conditions examined.

Uneven extension of specimens.

To overcome the difficulty of premature failure due to the jaws of the machine damaging the fibres of the specimen, a dumbell shaped specimen was used which was made in accordance with B.S.S. 1910. This configuration is ideal for materials such as steel which exhibit little extension, but in the case of material like skin, even the short wide end portions will extend an appreciable amount under the action of the tensions applied. This will produce a source of error as these extensions will be included in that of the actual test portion. The effects of this problem were minimised by ensuring that the specimen was mounted in the machine in such a way that the whole of the wide portion was gripped within the jaws (figure 65). This ensured that, apart from the test length, only a very short section where the change of width of specimen occurred was included between the jaws, and was less than 7% of the total length. The situation was further improved by the wide end of the tapered portion being held within the jaws in such a way that it could not contract in width. This prevented it from extending in some measure and it was noted that the

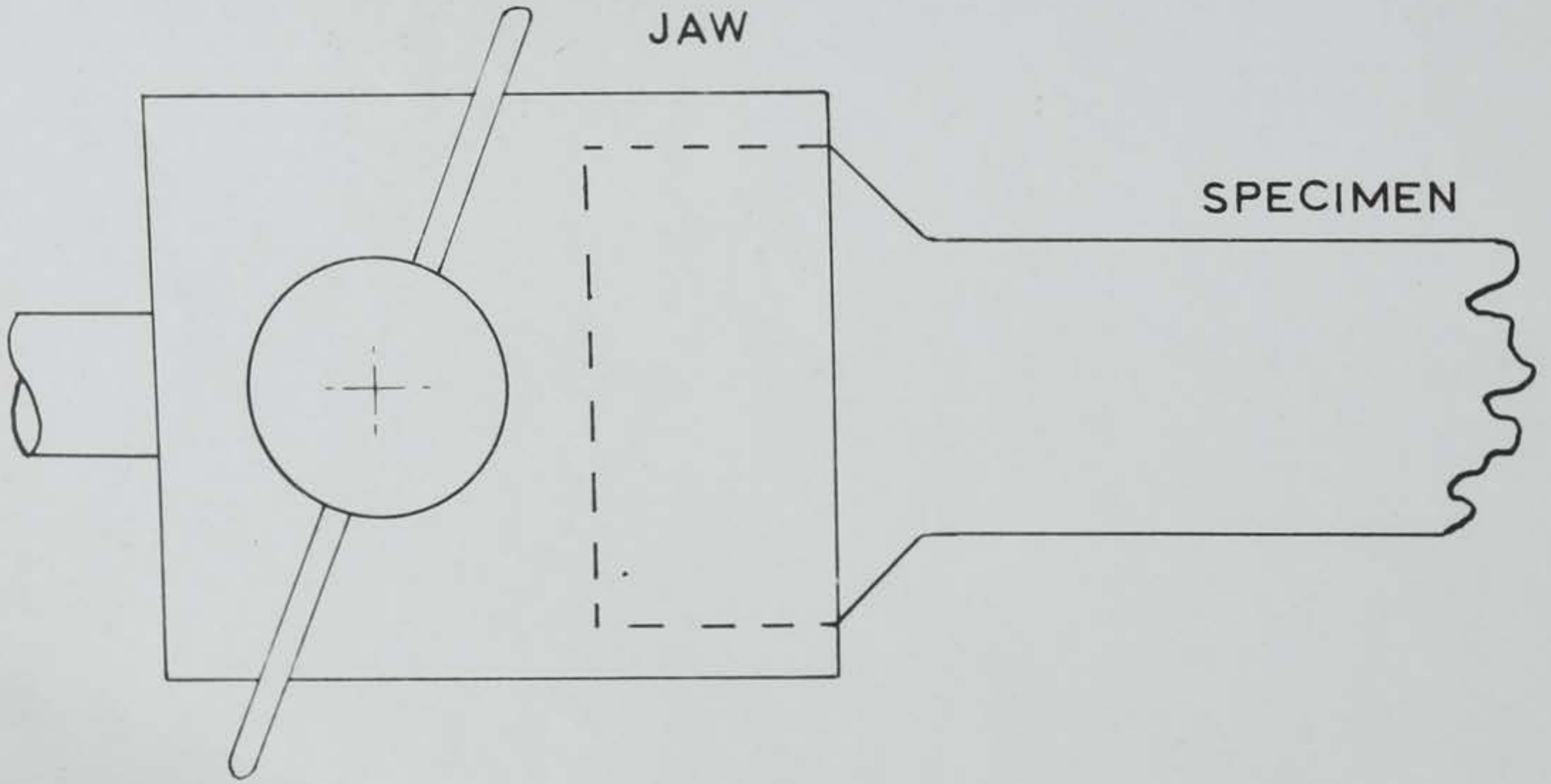


FIG. 65

POSITIONING OF SPECIMEN IN JAWS

configuration of the end portions of the specimen changed little irrespective of the amount of extension of the specimen.

Three dimensional model representing the rheological behaviour of skin.

The three dimensional system, although more difficult to handle mathematically than a normal type of two dimensional system, can produce an interesting and far more complete picture when expressed geometrically. Our analytical work mainly involved two dimensions, extension and load. However, a more pahoramic view may be obtained with the adoption of a third dimension, time. With a three dimensional system three sets of two dimensional systems can be produced, as seen in figure 66. The extension load (figure 66a) system is a familiar one which has been used throughout this work. The extension time system (figure 66b) concerns the results which can be obtained using a constant load apparatus and represents well known creep curves. These have been demonstrated in the present work and are well known in other biological tissues (Roy, 1880; Probine, 1959). The gradient of the straight line in the system, which is tangential to the curve or passing through the origin, will represent some velocity. The third system involves time and load and

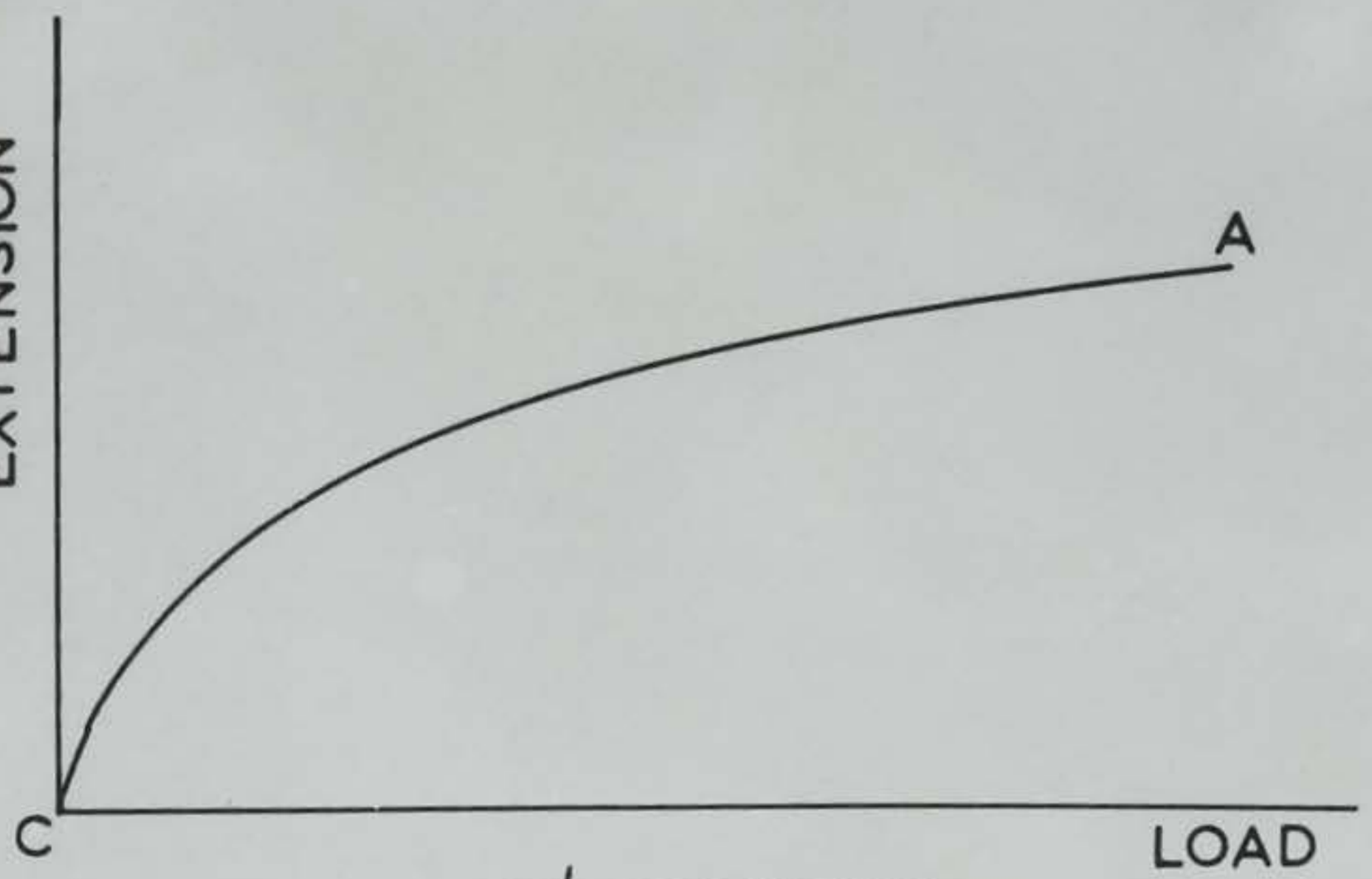


FIG. 66a LOAD/ EXTENSION

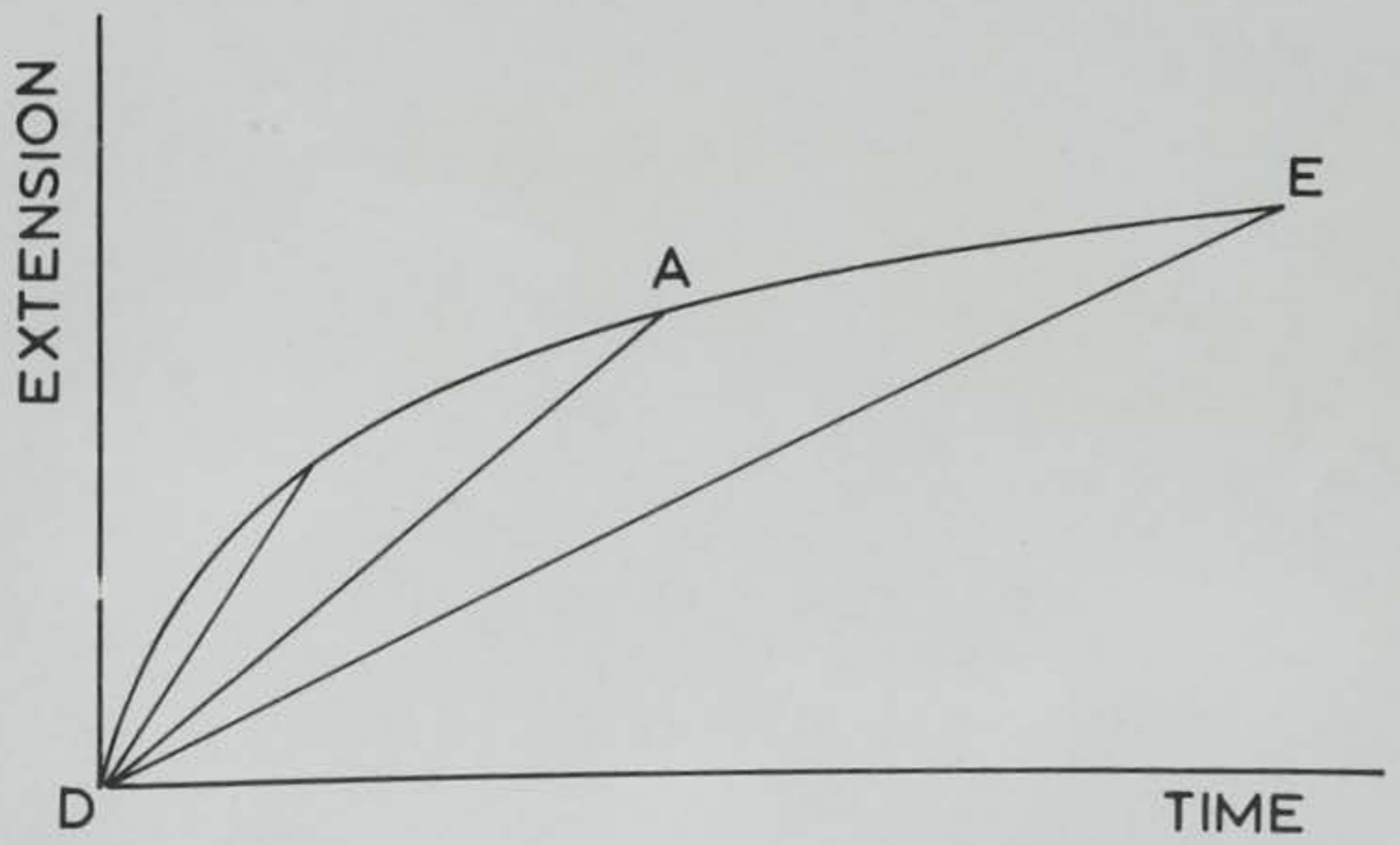


FIG. 66b CREEP

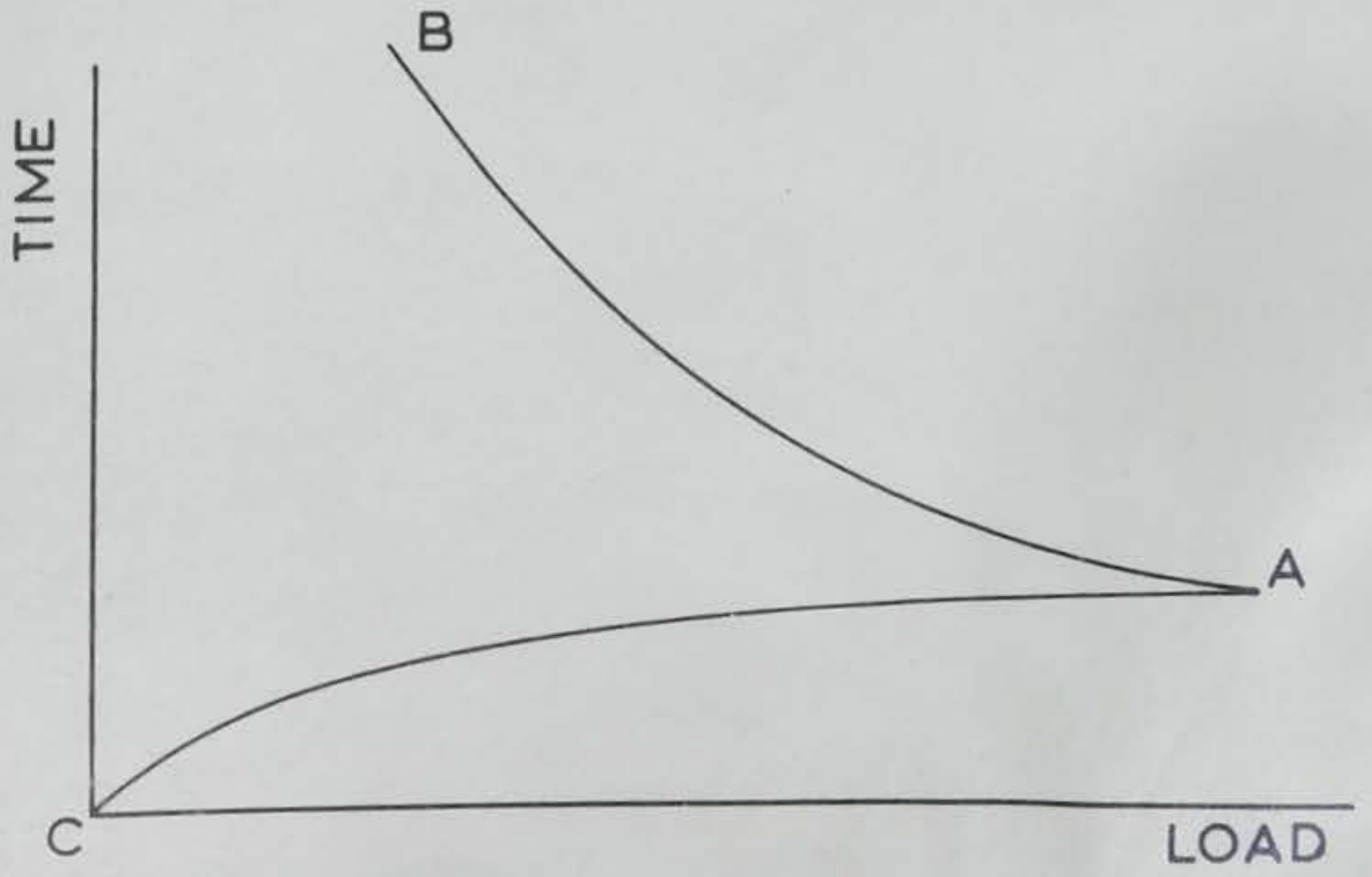


FIG. 66c RELAXATION

is the co-ordinate system on which stress relaxation phenomena are demonstrated, curve A B being typical of this (figure 66c). This has again been demonstrated in our work on human skin and has been noted in other biological tissues (Wood and Chamberlain, 1954; Ward and Popplewell, 1963).

These three co-ordinate systems may now be combined to represent the results in the form of a solid model (figure 67), which when viewed in the three directions X, Y and Z is seen respectively as the three co-ordinate systems. Considering now the normal extension process, the curve C A will be produced as shown in figures 66a, 66c and 67. It can be seen in figure 67 that this curve produces a plane with its load axis C D. When viewed in direction Y this plane appears as a line A D, as seen in figure 66b. Thus the angle at which this plane lies to the horizontal is controlled by the velocity at which the specimen is extended. A lower velocity of extension would produce the line D E for example on figure 66b. Thus, returning to figure 67, each different rate of extension may be considered to represent a plane at some particular angle to the horizontal. It can be seen that the slower the rate of extension the greater the extension achieved for a particular load. This is

a well known property of plastic material, and thus the solid represented by figure 67 can be assumed to consist of a whole series of planes at different angles. If the extension mechanism is stopped at some point A on figure 66c, relaxation will obviously take place. However, this is at a given extension and so the curve A B must be followed at a height above the horizontal corresponding to the initial extension. The curve A B can be seen more clearly on figure 67.

This concept while not yielding fundamental information regarding the structure of skin is useful for demonstrating the relationship between the various rheological phenomena observed. It can be seen that these are not isolated experimental findings, but all fit together to produce a complete picture of the whole situation.

Idealised representation of the structure of skin.

It has been shown that if a specimen of skin is subject to a progressive series of small pulsatile extensions a stress strain curve of the correct form is produced, even though considerable relaxation may take place between each pulse. This may throw light oh the actual extension mechanism of skin, since relatively few rheological models can duplicate this effect. The simplest system of this type is shown in figure 68a, where

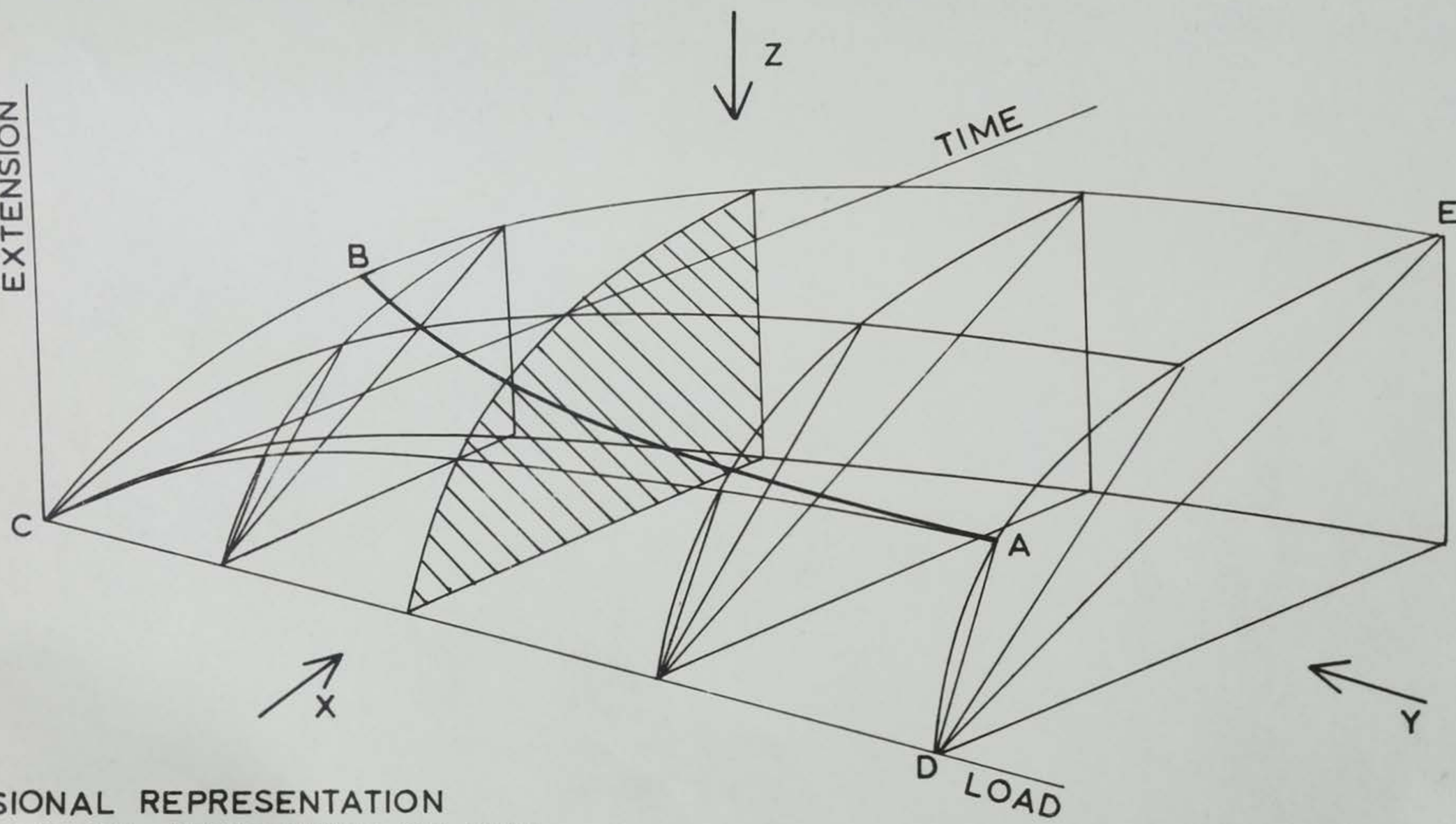


FIG. 67

3 DIMENSIONAL REPRESENTATION
OF RHEOLOGICAL PROPERTIES OF SKIN

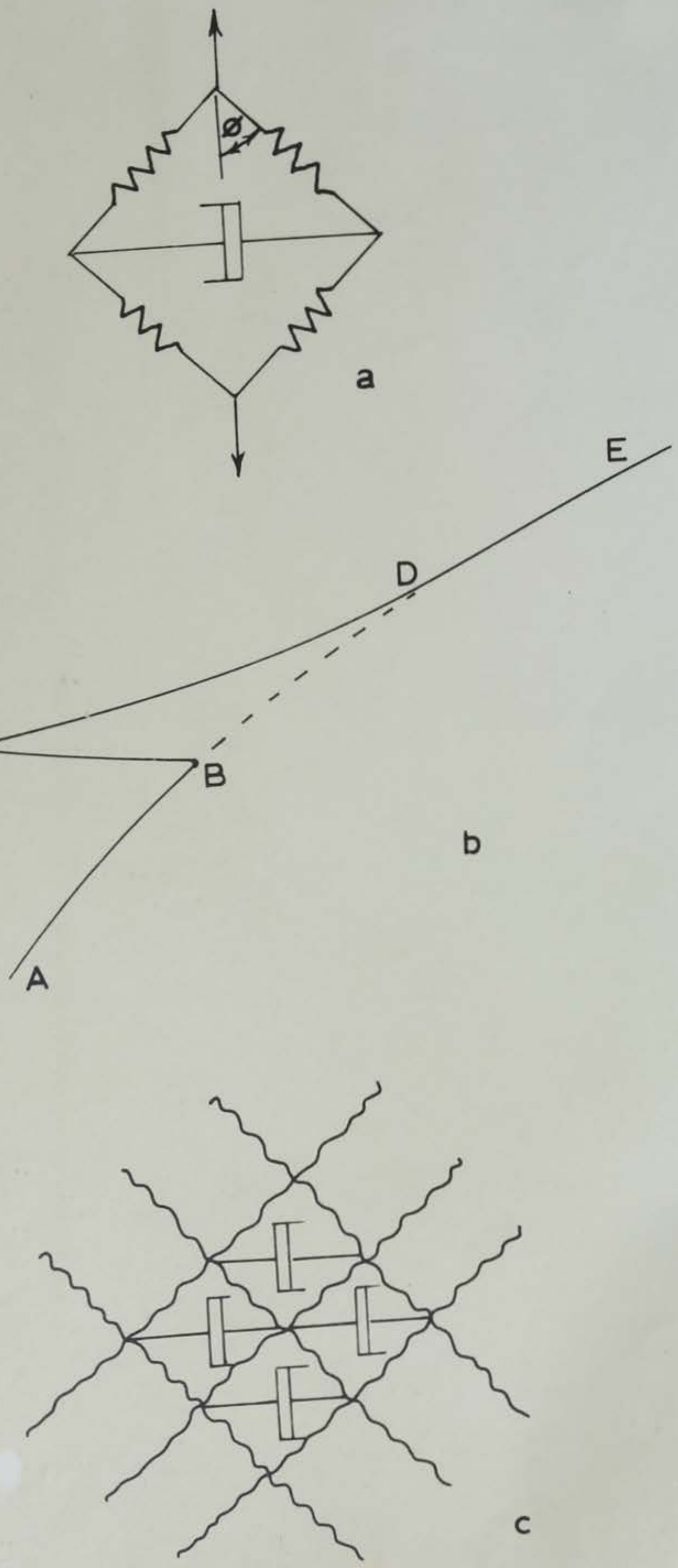


FIG. 68

DETAILS OF IDEALISED STRUCTURE OF SKIN

four springs are arranged in the form of a parallelogram and a dash-pot is attached between two opposite corners. If this system is extended at a constant rate, it becomes stiffer as the process progresses, because, as the dash-pot is moved, the angle ϕ between the springs becomes less, producing a greater component in the line of the pull. If the extension is halted at some point, the dash-pot continues to move and the angle ϕ becomes smaller. This produces a reduction in the tension of the system and corresponds to the stress relaxation observed in skin. When the extension is continued, the system behaves more stiffly due to the fact that the angle ϕ is less than it was when the extension was halted. However, now that the angle ϕ is less the horizontal component in the direction of the dash-pot is also less, and hence the rate of movement is lower, corresponding to a lower rate of change of stiffness. This represents the section C D in figure 68b. As the extension now proceeds as normal, but with a reduced rate of movement of the dash-pot, the situation will eventually be reached when the geometrical configuration of the system will be the same as it would have been had the extension not halted and had the resulting stress strain curve continued along the course of a normal extension, corresponding to D E. This system could be represented by one section of a lattice,

as shown in figure 68c, where the springs are represented by collagen fibres and the dash-pots by the ground substance between the fibres. This is obviously somewhat similar to the actual system, but the analogy cannot be carried too far as the behaviour of the ground substance and collagen fibres is far more complex than that of a simple dash-pot and springs. Nevertheless this type of reasoning has thrown light on such problems as deformation in porous rocks (Attewell, 1962).

Tests have been carried out in which the specimen was extended in a number of stages, permitted to rest and returned to zero between each extension process. It was found that on continuing the extension the stress strain curve resumed the course that it would have taken had the extension process not been halted. This process can be considered as an extreme case of the pulsatile system mentioned above. This phenomenon is useful in that it provides a means whereby the effects of subjecting a specimen to various experimental conditions may be observed. For example, a specimen may be partly extended and then subjected to enzymatic activity or freezing and the extension continued. If the curve for the second part of the extension had been plotted on semi-log paper

and the intercept became tangential with the line for the initial extension, it could be assumed that the activity has had no effect on the mechanical properties of the specimen. This has the advantage that the whole experiment can be carried out on a single specimen rather than two or more and thus the problem of the identical nature of a control specimen is eliminated.

Tests of this nature have been carried out on both skin and rat tail tendon collagen fibres to investigate the effect of freezing. The results demonstrated that freezing had no effect on the rheological characteristics. To obviate any errors introduced by the removal of the specimen and jaws from the machine during freezing, the whole experiment could be conducted with the tank on the machine. An alternative method of comparison of frozen and unfrozen specimens from contiguous sites confirmed the results obtained in the experiments in which rat tail tendon collagen fibres were extended, removed, frozen and then re-tested.

Poisson's ratio.

Poisson's ratio is the ratio of strains in the directions perpendicular to that of extension to that in the direction of extension (Love, 1934). For an iso-

volumetric extension the strain ratio of width to length should be 1.0. However, it has been shown (Kenedi, 1964 b) that the value for skin of this strain ratio can vary from 0.4 to 1.7 as the extension process progresses, the increase being rapid at first and levelling out subsequently. High values of Poisson's ratio for the skin of the guinea pig have been reported by Beckworth (1963). An extensive mathematical analysis of the properties of fibrous materials has been carried out by Cox (1951) who has shown from theoretical considerations, that for a solid planar mat the value of Poisson's ratio with respect to the thickness is very small and can be considered as negligible. Nevertheless, the Poisson's ratio with respect to width increases rapidly as extension progresses to values considerably in excess of unity. For the skin, more factors such as the properties of ground substance surrounding the fibres, would have to be taken into account to make such an analysis complete. A simpler explanation for this change of the strain ratio as extension progresses may be seen by considering a simple idealised portion of a lattice structure of semi-angle ϕ which is extended. It can be seen that if ϕ equals 45° an incremental extension will produce a reduction in width of a similar amount, giving a strain ratio of unity. By

similar reasoning it can be seen that ϕ is in excess of 45° the strain ratio will be less than unity. Conversely, as ϕ reduces from 45° to approach zero the strain ratio will increase to approach infinity. In practice, however, these excessive values are avoided, due to the finite thickness of the fibres, and the spaces between them being filled with ground substance and elastic fibres, preventing the meshwork closing completely. Moreover, the fibres themselves are extended as the process continues.

Characterisation of stress strain curve.

Polynomial expression.

One method of describing the stress strain curve produced by skin is by a polynomial expression (Hall, 1951) and an excellent result may be obtained if sufficient terms are taken, e.g.

$$E = a_o + a_1 L + a_2 L^2 + a_3 L^3 \ldots\ldots a_n L^n$$

where E = extension
L = load

$a_o\ a_1\ a_2 \ldots\ldots a_n$ = constants

In many cases an adequate result may be obtained by using only the first three or four terms of such an expression but more constants are usually necessary than those of the equivalent power law. Moreover it may be extremely difficult or even impossible to relate the various constants to physical properties of the specimen.

Equations used.

The extension of skin may be divided into two separate stages; the first represents the straightening and orientating of the fibres, and has been shown to be described by the equation

$$E = a + b \log L$$

where E = extension
L = load
a & b = constants

This phase ceases at approximately 100gm. (Ridge and

Wright, 1964). However, such results give no indication of the nature or quantity of collagen. It is possible that data concerning the properties of the ground substance could be evaluated by carrying out tests at different rates of strain and observing the change of gradient in this early part of the curve. Collagen is apparently not being extended in this range and work done on the present apparatus has shown the effect of elastin is not dependent on the rate of extension (Hall, 1964).

The second stage of the stress strain curve can be described by the equation:-

$$E = c + k L^{b}$$

where E = extension
L = load
c, k & b = constants

The suggestion that this represents orientated collagen is borne out by the fact that it is the same equation as that obtained for rat tail tendon in the present work and also in that of Wood (1954).

A similar equation for the second part of the curve has been found to apply to skin by Kenedi, Gibson and Daly (1964). Their power term was however applied to extension rather than load and is thus the reciprocal of that obtained in the present work. Calculation of the values of constant b at various ages from the published

results of Kenedi, Gibson and Daly (1964) shows that their results are comparable to those obtained in the present study (Table 21).

Such differences as exist in the values for the two series may well be due to the fact that the experimental conditions were not identical.

Age (years)	40	50	60	70	80
Kenedi, Gibson and Daly	0.33	0.27	0.22	0.19	0.15
Ridge	0.35	0.34	0.28	0.22	0.16

Table 21. Comparison of values of b calculated from the data of Kenedi et al (1964) compared with values for male abdominal skin in the present study.

The constant b.

b is a property of collagen dependent on its stiffness. A low value of b indicates a greater stiffness (i.e. a larger force is required to produce a given extension) a higher value of b indicates less resistance to extension. b has been shown mathematically to be a specific property of the collagen fibres, and is independent of the length, initial degree of orientation or number of fibres in a specimen. This fact has been demonstrated by finding that specimens of various widths gave similar values. Moreover, specimens tested along and across Langer's lines, where the fibre pattern is very different, also gave similar values in the two directions.

It has been shown that the more rapid the extension the lower the value of b. This would be expected, as "plastic" materials are stiffer at higher strain rates.

The constant k.

k is a composite term made of the quotient $\frac{l}{n^b}$ where l is the straightened, orientated fibre length, and n the area of collagen under load. The value of k decreases with age. Biochemically it has been shown that there is no significant difference in percentage collagen content dry-weight throughout the age range. From theoretical considerations therefore the value of n in a given direction must be constant with age. The reduction in the value of k with age therefore indicates a shortening of the fibre, probably due to increased cross-linkage and possibly to the fact that perfect orientation is restricted by increased amounts of inter-fibrillary material (Tunbridge, Tattersall, Hall, Astbury and Reed, 1952).

A greater value of k is obtained for specimens taken across Langer's lines than for those along. This provides supporting evidence for the lattice theory, from which it would be predicted that the value of l would be greater and that of n less across Langer's lines.

It is preferable to calculate the value of l from the sum of the "zero load" length and the value of c found by the computer, as this eliminates the need to solve two simultaneous equations for specimens taken along and across Langer's lines. Thus, by using this value of l, n may be found from k, enabling all the constants to be evaluated from a single specimen.

The value of l depends on the amount the specimen has shrunk when cut from the body. The greater the shrinkage, the higher the value of l. Consequently the true value of l is greater by this amount, which is approximately 10 per cent. Wenzel (1949) quoted a mean value of some 14 per cent for dermal shrinkage. The amount of shrinkage will also depend on the direction in which the specimen has been cut with reference to Langer's lines. Those cut across the lines will shrink less than those cut along the lines.

Variations with site.

It has been shown that the shape of the b/age curves were similar for all sites investigated with the exception of male forearms. In the case of females it was shown that the reduction in the value of b was less for the skin of the forearm than the abdomen, giving a decrease in b of 0.08 for forearm skin, compared with 0.30 abdominal skin over the same age range of 40 to 90 years. There was a

suggestion that the increase of the value of b from 0 to 40 years was similar in both sites (0.27 to 0.44). Results were inconclusive due to the difficulty in obtaining material in the lower age ranges. It has been shown that for all sites except male arms, the values of k and l decreased with age.

It would thus seem that in the case of females the changes due to ageing are less pronounced for forearm skin than for abdominal skin, both with respect to changes in the stiffness of the collagen (b) and the architecture of the wickerwork (k).

In the case of male arms, the scatter in the values was large. This may well be occasioned by environmental differences to which patients had been exposed. Some had their forearms permanently covered with clothing, while others were doing manual work with their arms exposed to sunlight and considerable mechanical action. There is evidence that sunlight not only contributes to the break-down of elastin but may also affect collagen in the same way to a lesser extent (White, 1910; Ejiri, 1936; Dick, 1947; Annotation, 1962).

Variations with sex.

It has been shown that there is a great similarity in the shape of the b/age curves for skin from male and

female abdomens. The only difference was that over the whole age range the value of b for females was 0.07 higher than that for males, signifying greater extensibility. Again, comparing male and female abdominal skin, values of l and k apparently decreased in a linear manner with age, but there was no difference between the sexes.

It would thus seem that the differences observed in the properties of male and female abdominal skin are due to changes in the collagen itself, rather than in the wickerwork.

It is interesting to observe that skin retraction when a specimen is removed from the body is greater in women than men. This may in fact be only indirectly related to sex, the true correlation being with the amount of subcutaneous fat, since limited data in the present study suggests that skin from obese subjects retracts more than that from thin subjects.

Changes in the stiffness of collagen with age.

The variation of the stiffness of skin with age found in the present study agrees well with the findings of Harkness (1964) in rabbits and Kenedi, Gibson and Daly (1964) in man. These differences are statistically significant, particularly with abdominal skin. The most likely explanation of the increased stiffness with advancing age after 40 years is that the number of cross-links in the collagen fibres has increased.

It is clear from the biochemical studies that the increased stiffness is not due to a greater percentage of collagen in the skin with advancing years . Verzar (1963) stated that collagen fibres once synthesised are permanent and remain for the rest of life, but are nevertheless subject to a maturing process, in which the number of cross-links increase. This is somewhat at variance with the findings of Neuberger and Slack (1953) and Slack (1954a) who believe that the turnover of collagen, while being very slow, is completed in a cycle of approximately 15 years. There are well known exceptions to this, e.g. in the carragenin induced granuloma (Jackson, 1957), the pregnant uterus (Harkness, 1961), and the rat limb stripped of skin and enclosed in the animal's abdomen (Slack, 1954b). In adult life however the turnover of collagen is undoubtedly slow (Slack, 1954a). It is possible that with advancing years the skin contains a progressively higher proportion of older collagen which will contain a greater number of cross links. Strong evidence to support the cross linking theory is that the tensile strength of skin increases with age (Rollhauser, 1950). The present work provides confirmatory evidence of this, since the points of inflection of difference distribution systems (an indication of the beginning of the yielding process) appear to be higher with increasing

age. This has also been demonstrated by Verzar (1957), investigating the thermal shrinkage of collagen fibres.

This theory explains the increase in stiffness with age, but does not explain the increase in the value of b up to the age of 35 or 40. This implies a reduction in stiffness. The work of Whowinkle (1933) provides support for this suggestion. He investigated samples of facial skin in the age range of 5 to 101 years and formed the opinion that senile alterations commenced in the fourth decade starting in the epidermis and extending down into the dermis with advancing years. Alterations involved a degeneration of both elastic and collagenous fibres and resembled those caused by Roentgen rays and radium.

Tate (1964) working in our department has investigated the problem of the burst abdomen by subjecting sections of sutured aponeurosis to very rapid extensions and observing the loads required to rupture the specimens. He has demonstrated that in similar conditions specimens require a progressively greater load with increasing age up to 40 years. After this age the situation is reversed and the load required to rupture the specimen diminishes with age. This is unexpected in the light of the facts that the tensile strength of tissue increases

with age (Wohlisch, 1927) and that the present work has demonstrated that the stiffness of skin increases with age. However, it must be remembered that the property investigated by Tate is probably not pure tensile strength. It may be that on applying a load to a relatively extensible specimen the tissue forms round the sutures in such a way that stress is distributed more evenly, enabling a greater total load to be tolerated. In the case of a stiffer specimen, less deformation would be produced and more acute stress concentrations would be formed in the area immediately around the suture, causing a premature failure out of proportion to the tensile strength of the material.

The effect of age on the elasticity of skin.

It has been said "when the skin of a relaxed hand of a medical student is pulled up between the finger and thumb and released, it snaps back into place, whereas that of the senile instructor subsides slowly and with dignity because the elasticity is reduced" (Ma and Cowdry, 1950). This observation demonstrates one of the most significant changes taking place in skin with increasing age. This fact can further be demonstrated by the shrinkage which takes place in a piece of skin which is removed from the body. Dermal shrinkage has been investigated by Evans, Cowdry and Neilson (1943), who observed

that it was decidedly more vigorous ih young persons than in old ones. This has also been demonstrated in our own experiments by cutting out inch squares. Dick (1951) stated that the natural tension in skin was greatest at points where the elastic meshwork was greatest. He also noted that the contraction of a piece of skih when removed from a site of dense elastic meshwork was greater than would be obtained from a piece of skin taken from a site where the meshwork was less dense. It would thus seem that the strong elastic meshwork causes specimens to contract. However there has been difference in opinion as to which component of the skin produces the elasticity. Lynch (1934) was of the opinion that the collagenous meshwork contributed the greater component. However this has been refuted by numerous workers. Rothman (1953) observed that old people's skin has a poor elastic meshwork producing low initial tension. Schmitt (1891) referred to the breakdown of elastic tissue with age. These findings are also confirmed by Ejiri (1937a) and Dick (1947). Wenzel (1949) observed that the contraction of skin is greater in the direction of Langer's lines than across it.

This skin tension has also been verified during the present work in which inch squares were excised and it was noted that contraction occurred asymmetrically, that

along Langer's lines exceeding that across by as much as 25%. Waterton (1931) believed that the very existence of Langer's lines was due to the elastic meshwork. This favours the view that the tension in skin is caused by the existence of elastic fibres. However, it is conceivable that some part of the tension in skin could be due to the collagenous meshwork. Although the collagen fibres are relatively stiff and inextensible, they have their interstices filled with a gelatinous compound which will impart elasticity to the structure. It is also known that the elasticity of collagen decreases with age (Verzar, 1957) and that the natural tension is greater in the direction of the maximum number of collagen fibres. It could thus be that a considerable part of cutaneous elasticity is caused by a very small number of fibres which happen to be straight and completely orientated in the direction of extension.

Indeed Kenedi (1964b) has produced histological evidence, using the Mallory trichrome stain (which selectively stains collagen fibres red instead of green if they have been strained), that some fibres rapidly come under load when small extensions are applied. One would expect these fibres to rupture first. At this time

increasing numbers of fibres would be orientated and available to carry the load. If this was the case it would be one explanation for the phenomenon of permanent set, since the original fibres would no longer be capable of pulling the specimen back to its original shape. Due to the drastic redistribution of ground substance the elastic fibres may be no longer capable of completely performing this function themselves.

It would thus seem that the natural tension in skin may be due partly to the effect of the elastic tissue and partly to the collagenous, possible the latter predominating in situ. On removal of a piece of skin from its habitat the collagen fibres rapidly come under stress with quite a small contraction because of their natural stiffness (which is however less for small strains) and the final part of the contraction may be due almost entirely to elastic fibres which being much less stiff than collagen will be capable of remaining under tension after considerable contraction. This would tend to support the theory developed by Tunbridge, Tattersall, Hall, Astbury and Reed (1952) that it is the collagenous structure which plays the major part in the elasticity of skin. It seems unlikely that the suggestion of Sternberg (1925) that the elasticity of skin is due to the collagenous framework and over distension is prevented by the elastic fibres is

correct, since the elastic meshwork is sparse compared with that of collagen and also its tensile strength is considerably less (Hall, 1964).

The directional shrinkage effect of skin has also been demonstrated in our work with the slab cutting technique, when the relatively large pieces of skin (e.g. 6 inches by 3 inches) have been used. Specimens cut from this with the die in the direction of Langer's lines were shorter than those cut across. This was due to the fact that the skin was mounted with a large amount of fat and subcutaneous tissue attached to it preventing full contraction before it was frozen into place.

Rothman (1953) noted that skin from the aged offered little initial resistance to stretching. This was readily demonstrated in the present work (figure 69). The initial portion of the stress strain curve was tangential to the extension axis. In skin from a younger individual there was considerably more resistance to the initial extension and the curve relatively rapidly separated from the extension axis (figure 70). It was thus seen that in the case of the aged where the specimen had contracted relatively little that the initial part of the curve was concerned with the orientating of the collagen fibres and the

FIG. 69
CURVE FOR ABDOMINAL SKIN OF 3½ MONTH MALE

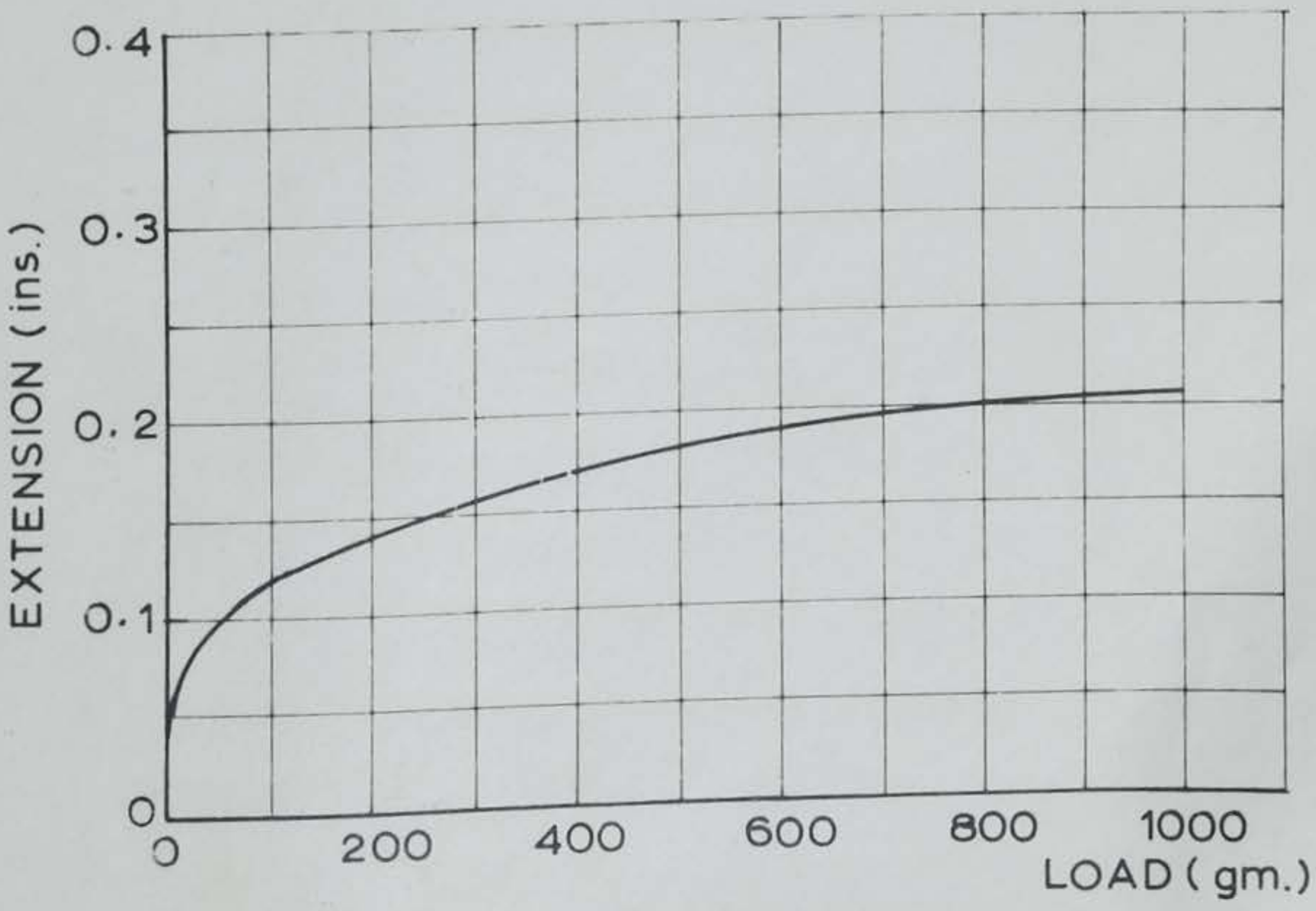


FIG. 70
CURVE FOR ABDOMINAL SKIN OF 82 YEAR MALE

process met with little resistance from the elastic meshwork. In younger individuals, considerable contraction had taken place due to a strong elastic meshwork which had somewhat compressed the collagenous meshwork. Consequently on extension the initial part of the curve was concerned mainly with stretching the elastic meshwork. A considerable extension was required before the collagen fibres were fully orientated.

Collagen content.

The percentage of collagen in skin was determined for the following reasons.

1) As an independent check that the value of the constant b did not depend on the amount of collagen.

2) To determine whether the constant k varied as predicted.

3) To determine whether changes associated with various connective tissue disorders were accompanied by variations in the amount of collagen.

4) So that it might be possible if necessary to express the strength of skin in terms of load per unit of collagen.

Harkness and Harkness (1961) in their work on the cervix of the rat with relation to pregnancy used the method outlined in section four. However in the case of

skin, it was found that the percentage collagen varied so little that it was easier where necessary to express the results in terms of stress based on the cross-sectional area of the specimen.

A mean value of 65.6% collagen dry-weight was found for both male and female skin throughout the whole age range. Rothman (1953) quoted a value of 71.9% for the collagen content of skin, but did not specify the site from which it was taken. In the present study skin from the arms had a value of approximately 10% less than the abdominal skin for both sexes. It is of interest to note that a value of 77% of collagen for dry skin has been obtained by Wernstein, Weinstein and Bouckek (1960). This was for skin stripped of its epidermis which contains little collagen. It is possible that the reduced value found for skin from the arms may be due to the fact that the epidermis is thicker on the arms than on the abdomen as a result of exposure to sunlight and mechanical action.

It has been shown that for a given site and sex the percentage of collagen in the skin remains virtually constant at all ages. It follows therefore that the value of the constant b, which has been shown to vary considerably with age, is not dependent upon the collagen content. In view of the finding of this constant

collagen content of skin irrespective of age it may be deduced that the constant n is similarly unrelated to the age of the subject. The results show that the constants l and k are related to each other over the full age range and provide confirmatory evidence for the proposed lattice theory.

In the small number of specimens examined in patients with inflammatory polyarthritis the collagen content was somewhat lower than in skin from subjects unaffected by arthritis, matched by sex and age. Although these disorders are sometimes termed "collagen diseases" (Klemperer, 1953) little is known about quantitative or qualitative alterations in the collagen.

Disorders affecting collagen.

A rheological study of skin will provide information about connective tissue in those disorders which produce alteration in it throughout the body. For instance, in Ehlers Danlos syndrome there are obvious clinical alterations in the distensibility of skin. This rare disease has attracted much interest over the years, so that McKusick (1960) quoted 152 references to it over a period of 300 years. Yet the basic pathology is still not conclusively elucidated. Wright and Johns (1960) using

rheological techniques in the evaluation of joint stiffness produced evidence in a single case that the major alteration may be in the ground substance.

Recently in the controversy on whether osteoporosis is primarily due to a calcium deficiency or a defect in the collagen matrix clinical evidence has been produced to suggest that there is indeed a generalised alteration of collagen, affecting the skin as well (McConkey, Fraser, Bligh and Whiteley, 1963). This finding could be investigated further by the use of the techniques developed in this study. In a single case of osteoporosis on which rheological studies could be done no significant differences in collagen content were found, and the interpretation of the difference in the constant k is difficult in a single case. The patient was an unusual example of osteoporosis occuring in a young woman after pregnancy. Those described by McConkey et al (1963) were patients with post-menopausal or senile osteoporosis.

A small number of patients with rheumatoid arthritis were investigated. The constant k was definitely increased in the three specimens of abdominal skin which were tested. The collagen content of skin in three patients appeared to be reduced. The patient who showed

the biggest reduction (the lowest value obtained in any estimation) also showed the greatest difference in the constant k. The difference was too great to be attributed to the change in the constant l and must therefore have been due to a reduction in the value of the constant n. The value of constant b was reduced indicating increased stiffness. The reduction in the value of b was a consistent finding in skin taken from six sites in four patients with rheumatoid arthritis. The explanation would seem to be that there was less collagen, but that it was stiffer probably due to increased cross-linking. The interpretation of the significance of these results must however be tentative at the moment, since some of the patients were on corticosteroid therapy. This itself is thought to affect collagen (Herrick, 1945; Chieffi, 1950).

In animal experiments, Frey, Harkness, Harkness and Nightingale (1962) have investigated the tissues of lathyritic rats and chickens and shown that the collagen was unchanged but the tensile strength was considerably reduced in rat aorta, chicken skin and rat intestine. The extensibility was normal for rat skin and intestine, but greater for chicken skin. Protein depleted rabbits have been used in the present work to observe the effect

of protein depletion on the rheological characteristics of skin. This was done in parallel with work on the impact load on sutures in aponeurosis of these animals (Tate, 1964). It was found that protein depletion reduced the thickness of skin and aponeurosis but made no difference to the values of the constants obtained, indicating that the amount, the meshwork and the cross-linkage of collagen fibres was unchanged both in the skin and aponeurosis.

VII. SUMMARY

A critical analysis of rheological techniques as applied to biological tissues has been made. The structure, physiology and mechanical properties of skin and its components have been reviewed. A survey has been made of previous theoretical approaches to the structure of collagen.

To determine the rheological properties of skin initial studies were done with an Instron tester. An extensometer was built, utilizing a constant rate of extension system. The rate of extension could be varied and results were automatically recorded in the form of a stress strain curve from specimens obtained at autopsy and by biopsy from the forearm and epigastrium. Experiments were performed at a controlled temperature and the machine was modified as required to measure stress relaxation, stress recovery and tensile strength.

Specimens were cut to a constant thickness of 0.1 inches and were cut to a standard test shape with a dumbell shaped die. Rigidity was achieved by freezing prior to cutting (it was ascertained that this did not alter the rheological characteristics). Skin exhibited stress relaxation, stress recovery, permanent set and yielding.

The extension of skin fell into two main sections, each characterised by an empirical mathematical equation. The initial stage of extension produced straightening and orientation of the fibrous material (mainly collagen). The process was governed by the equation

$$E = a + b \log L$$

where E = extension
L = load
a and b = constants

The second stage of extension caused stretching of collagen fibres and was described by the equation

$$E = c + k L^{b}$$

where E = extension
L = load
c, k & b = constants

An equation of this form was found to characterise the extension of orientated collagen derived from rat tail tendons. The constants of the equation were accurately determined by use of a computer. The Taylor's series expansion method was utilised. It was necessary to select a specific range for each specimen, to obtain results with the minimum of distortion caused by the orientating and yielding effects.

The significance of the constants in the equation have been determined mathematically, and it has been shown that

"b" is a function of the stiffness of the collagen fibres, and is independent of the size of the specimen, "c" and "k" have been shown to represent the conditions of the fibre network, "k" being governed by the length and total area of the fibres. The validity of this system has been verified by experiments along and across Langer's lines and by varying the width of specimens. It has been shown that the constant b when plotted against age produces a curve which reaches a peak in the region of 40 years. This may well be due to the turnover of collagen. The constant k has been shown to be reduced with age. From changes in the value of b, it is seen that female collagen is more extensible than male, and that the stiffening process with age is not so rapid in skin from the arm as the abdomen.

Langer's lines have been verified in the sites studied and shown histologically and experimentally to be due to a preferential direction of fibre orientation. This has been investigated in relation to dermal shrinkage on removal of specimens. The percentage of collagen in dry, fat-free skin was ascertained by a modification of the method of Neuman and Logan (1950). There was no significant variation with age or between sexes for a given site. The values were less for skin from the arm than the

abdomen (mean 61.1% ± 3.7 and 69.6% ± 4.41 respectively).

Data on the effect of inflammatory polyarthritis are given and the effect of protein depletion in rabbits was studied.

The significance of the constants in relation to the structure of skin is described. The structural alterations produced by physiological and pathological conditions (with particular reference to the effect of ageing) and reflected in the rheological constants are discussed.

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APPENDIX 1

STANDARDISATION FORMULAE

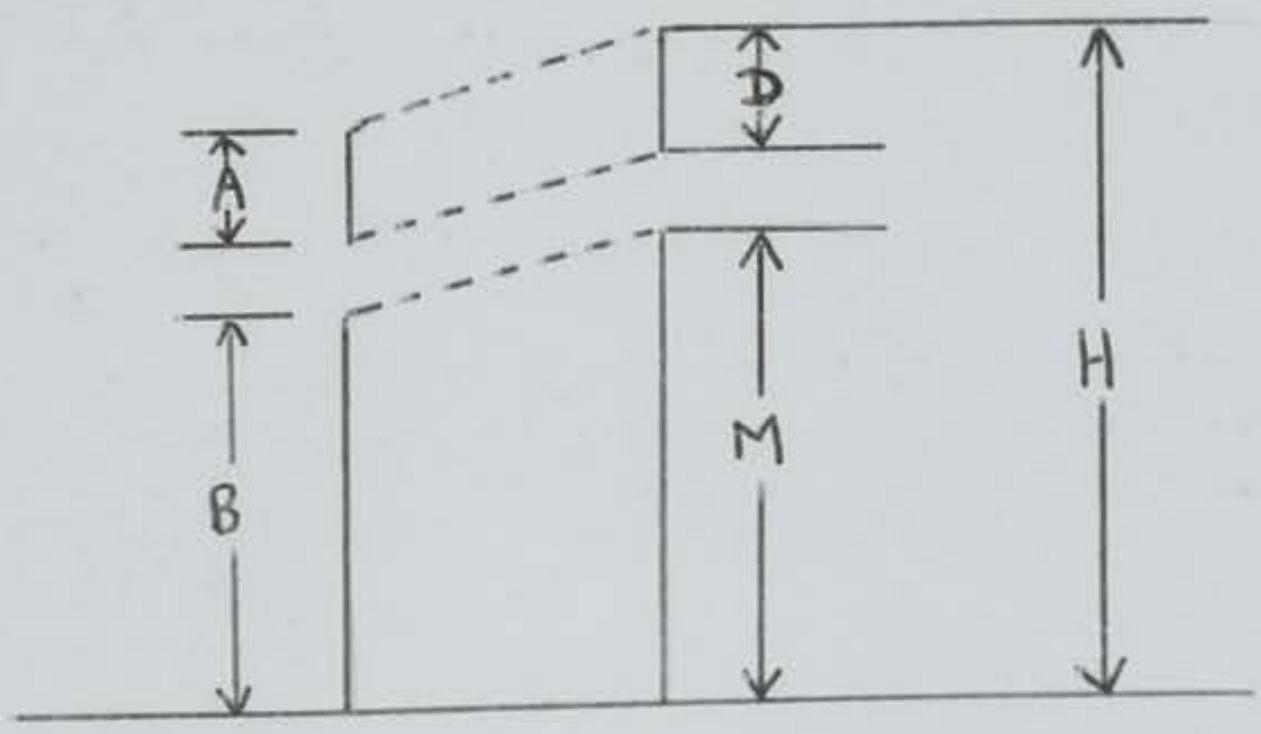
Let B = true gauge length

A = some extension of gauge length

M = actual initial length of specimen

D = extension of specimen by the same load which produced A

H = total extension of specimen



By proportion $D = \frac{MA}{B}$

$$H = M+O$$

$$H = M\left(1+\frac{A}{B}\right)$$

If specimen is shorter than standard gauge length this is

$$H = M\left(1-\frac{A}{B}\right)$$

APPENDIX 2

MALLORY TRICHROME STAIN (Modified)
(details supplied by Professor R.M. Kenedi)

Staining Solutions:

A.	Ponceau 2R.	0.2gm.
	Acid fuchsin.	0.1gm.
	Orange G.	0.1gm.
	0.2% Acetic acid in Aq. dest.	300cc.
B.	Light green	0.5gm.
	0.2% Acetic acid in Aq. dest.	100cc.

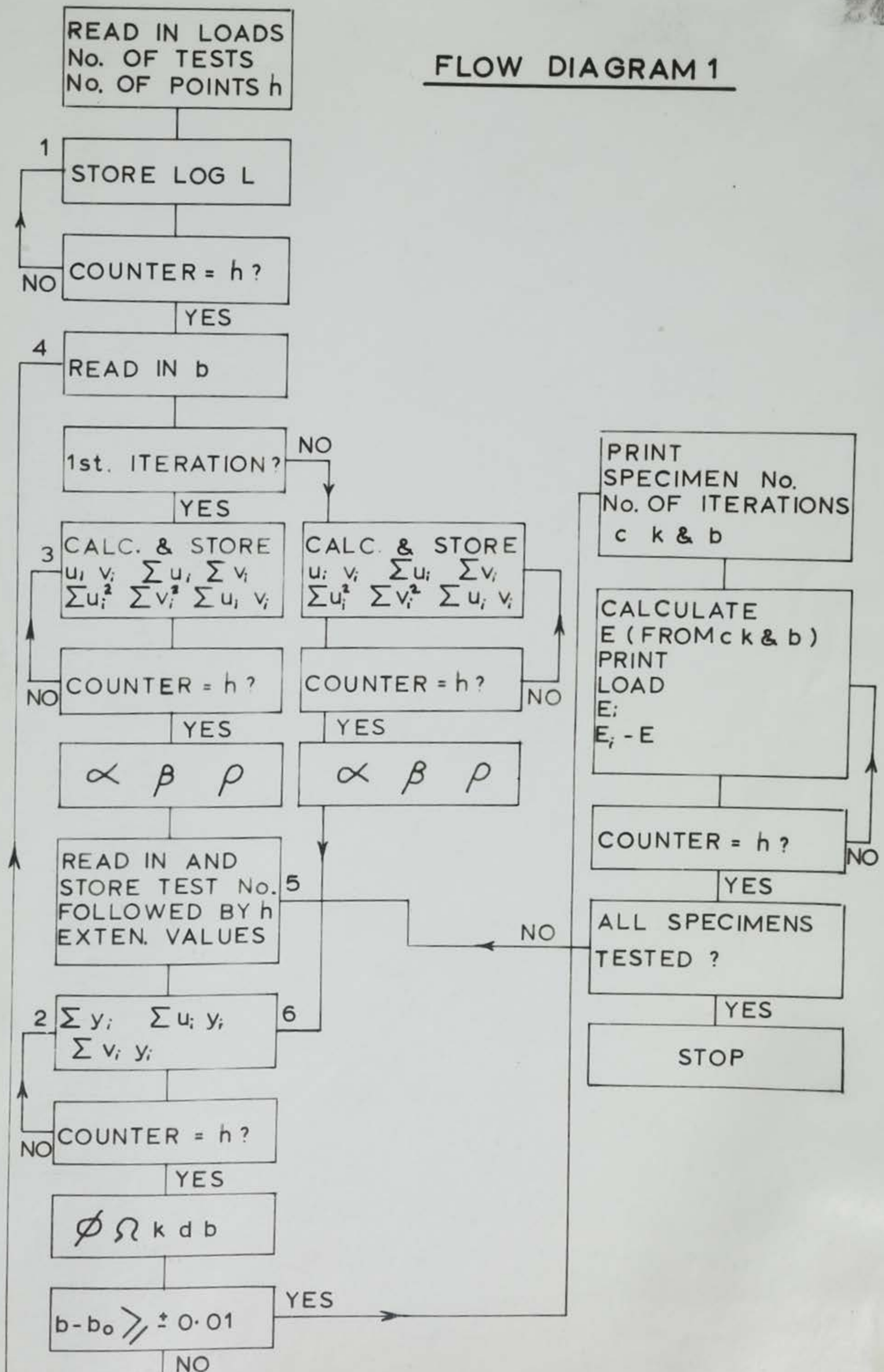
Method:

1.	Section to water. Treat artefact if necessary.	
2.	Celestin blue	5 mins.
3.	Rinse water.	
4.	Haemalum.	7 mins.
5.	Rinse water.	
6.	Ponceau mixture (Soln.A)	5 mins.
7.	Rinse water	
8.	0.2% Acetic acid	5 mins.
9.	Rinse water.	
10.	5% Phosphotungstic acid in water	5 mins.
11.	Light green mixture (Soln.B).	5 mins.
12.	Rinse water	
13.	0.2% Acetic acid.	5 mins.
14.	Dehydrate, clear and mount.	

Results:

Epithelial cells, muscle, fibroblasts.	Red.
Collagen and basement membranes.	Green.
Erythrocytes.	Orange.
Nuclei.	Purple to Red.

FLOW DIAGRAM 1



APPENDIX 4.

4. COMPUTER PROGRAMME I.

```
Q7890

N
STRESS STRAIN CONSTANTS
       N1        N2        A         B         C

STOP
n7=TAPE
n1=TAPE
v40=TAPE(n1+1)
v39=v40
v0=n1
n4=0
n0=0

1)v(100+n4)=LOGv(41+n0)
n0=n0+1
n4=n4+1
→1,n0≠n1
v90=0
v89=0
v88=0
v87=0
v92=0
n3=0
n8=0

4)v1=0
v2=0
v3=0
v4=0
v5=0
v6=0
v8=0
n4=0

3)v(400-n3+n4)=EXP(v39×v(100+n4))
XPv(240-n3+n4)=v(100+n4)×v(400-n3+n4)

v(87-n3)=v(87-n3)+v(400-n3+n4)
v(88-n3)=v(88-n3)+v(240-n3+n4)
v(89-n3)=v(89-n3)+(v(400-n3+n4)×v(400-n3+n4))
v(90-n3)=v(90-n3)+(v(240-n3+n4)×v(240-n3+n4))
v(92-n3)=v(92-n3)+(v(400-n3+n4)×v(240-n3+n4))

(
```

```
n4=n4+I
→3,n4≠nI
v(9I-n3)=v(89-n3)-(v(87-n3)×v(87-n3))/v0
SPv(93-n3)=v(92-n3)-(v(87-n3)×v(88-n3))/v0
v(94-n3)=v(90-n3)-(v(88-n3)×v(88-n3))/v0

→6,n3≠0
5)v600=TAPE(nI+I)
6)n2=0

n4=0
vII=0
vI2=0
v2=0
2)vI=v(60I+n2)
v2=v2+vI
vII=vII+(vI×v(400-n3+n4))
vI2=vI2+(vI×v(240-n3+n4))
n4=n4+I
n2=n2+I
→2,n2≠nI

vI3=vII-(v(87-n3)×v2)/v0
vI4=vI2-(v(88-n3)×v2)/v0

vI5=(vI3-((v(93-n3)×v(93-n3)×vI3)-(vI4×v(9I-n3)×v(93-n3))
)/(v(93-n3)×v(93-n3)-v(94-n3)×v(9I-n3)))/v(9I-n3)

n8=n8+I

vI6=(vI3×v(93-n3)-(vI4×v(9I-n3)))/(v(93-n3)×v(9
3-n3)-(v(94-n3)×v(9I-n3)))

SPvI8=(vI6/vI5)+v39
vI7=vI8-v39
v39=vI8
n3=84
→4,MODvI7≥0.0I
v39=v40
vI9=(v2-(vI5×v(87-n3))-(vI6×v(88-n3)))/v0

PRINTv600,3I60
PRINTn2,4I40
PRINTvI9,4084
PRINTvI5,4084
PRINTvI8,4084
PRINTn8,4080
```

n_3=0
7)v_{700}=(v_{15}XEXP(v_{18}Xv(100+n_3)))+v_{19}
v_{701}=v_{700}-v(601+n_3)
PRINTv(41+n_3),3080
PRINTv_{700},4006
PRINTv_{701},4006
n_3=n_3+1
⇒7,n_3≠n_1
n_8=0
n_3=0
n_7=n_7-1
⇒5,n_7≠0
(⇒0)

APPENDIX 5.

5. COMPUTER PROGRAMME 2.

(RANGE SELECTION)

```
Q7890
v80=TAPE*
STOP

11)v40=TAPE20
n6=0
v2=0
n3=0
n4=0
v3=0
v4=0
n5=19

n7=0
2)v1=v(41+n4+n3)
→11,v(160+n7)=0
v2=v2+v1
v3=v3+(v1xv(120+n4+n3))
v4=v4+(v1xv(140+n4+n3))
n4=n4+1
→2,n4≠n5

n8=0

n4=0
v0=n5
v5=v3-(v(160+n7)xv2)/v0
v6=v4-(v(161+n7)xv2)/v0

v7=(v5-((v(166+n7)xv(166+n7)xv5)-(v6xv(165+n7)
xv(166+n7)))/(v(166+n7)xv(166+n7)-v(
167+n7)xv(165+n7)))/v(165+n7)

v8=(v5xv(166+n7)-(v6xv(16
5+n7)))/(v(166+n7)xv(166+n7)-(v(167+n7)xv(165+n7)))

XPv9=v8/v7+0.3

v10=(v2-(v7xv(160+n7))-(v8xv(161+n7)))/v0

→15
16)v0=n5
n4=0
```

```
v23=0
v24=0
v33=0
v34=0
v35=0

13)v21=EXP(v9xv(100+n4+n3))
v(500+n4)=v21
v22=v(100+n4+n3)xv21
v(550+n4)=v22

v23=v23+v21
v24=v24+v22
v33=v33+(v21xv21)
v34=v34+(v22xv22)
v35=v35+(v21xv22)

n4=n4+1
→13,n4≠n5

v36=v33-(v23xv23)/v0
v37=v35-(v23xv24)/v0
v38=v34-(v24xv24)/v0

n4=0
v1=0
v2=0
v3=0
v4=0

14)v1=v(41+n4+n3)
v2=v2+v1
v3=v3+(v1xv(500+n4))
v4=v4+(v1xv(550+n4))
n4=n4+1
→14,n4≠n5

v5=v3-(v23xv2)/v0
v6=v4-(v24xv2)/v0

v7=(v5-((v37xv37xv5)-(v6xv36xv37)
)/((v37xv37)-(v38xv36)))/v36

v8=(v5xv37-v6xv36)/(v37xv37-v38xv36)

XPv9=v9+v8/v7
v10=(v2-(v7xv23)-(v8xv24))/v0
```

```
I5)vII=v8/v7
n8=n8+I
→I7,n8=4
→I6,MODvII≥0.02
I7)v2=0

v2=0
v3=0
v4=0

n4=0

→I2,n3≠0
→I6,MODvII≥0.001

PRINTv40,3060
I2)PRINTv(80+n3)XI0000,3060
PRINTv(80+n3+n5-I)XI000,4060
PRINTn5,4060
PRINTvI0,4043
PRINTv7,4043
PRINTv9,4043
PRINTn8,4020
→9,n3≠0

n2=0
4)vII=(v7XEXP(v9Xv(I00+n2)))+vI0
vI2=vII-v(4I+n2)
PRINTv(80+n2)XI000,3040
PRINTvI2XI0000,4040
n2=n2+I
→4,n2≠I9
n3=I
n5=7
n7=I0
n6=I
→2,n2=I9

9)n6=n6+I
v20=n6
v2I=v20XI0
n7=v2I
→5,n3=I
→6,n3=2
→7,n3=3
→8,n3=4
→I0,n3=5
```

5) n5=n5+1
→2, n5≠12
n5=5
n3=2

6) n5=n5+1
→2, n5≠12
n5=5
n3=3

7) n5=n5+1
→2, n5≠12
n5=5
n3=4

8) n5=n5+1
→2, n5≠12
n5=5
n3=5

10) n5=n5+1
→2, n5≠12

→11
(→0)

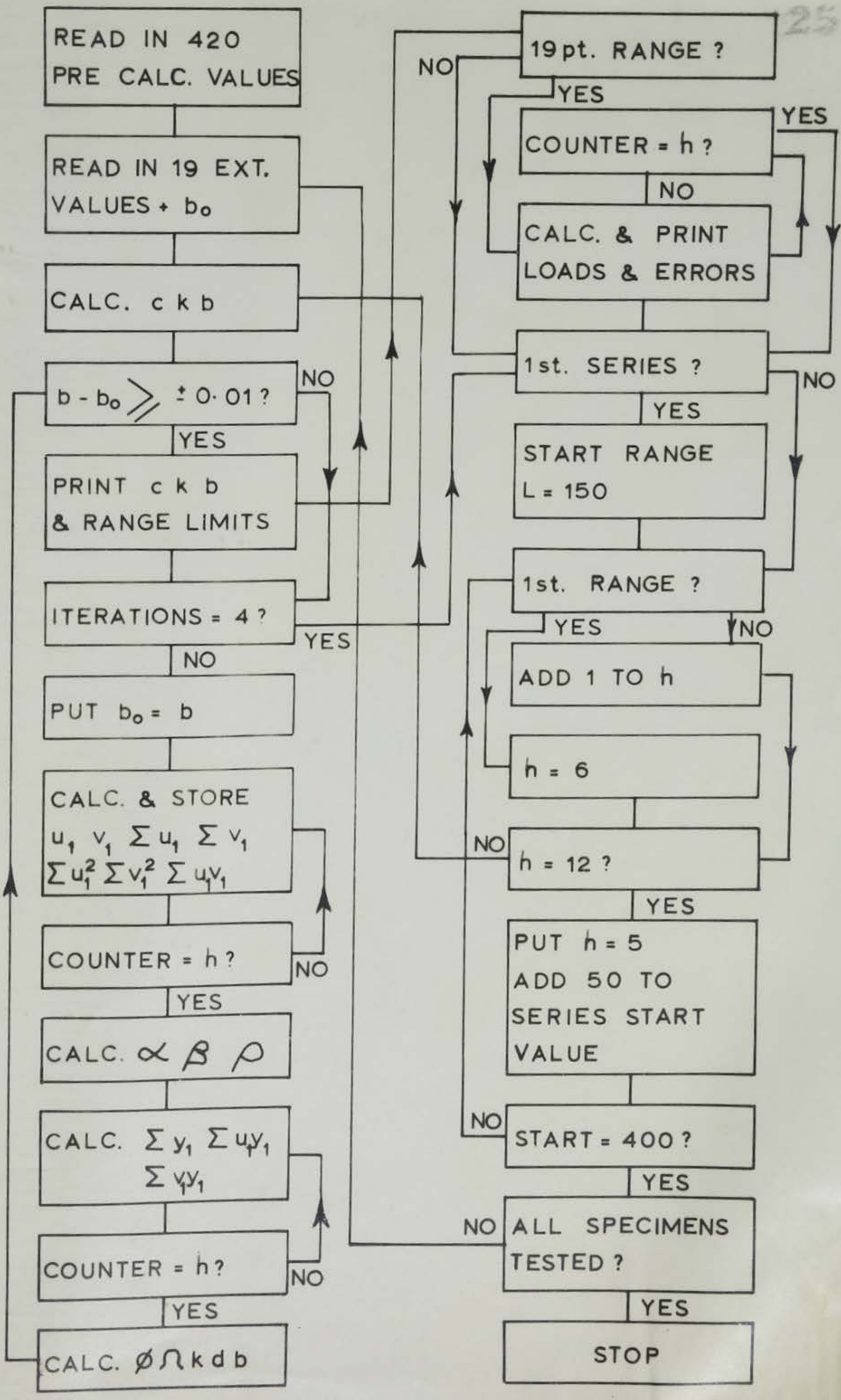
6. ANCILLARY PROGRAMME

(LOAD BLOCKS)

```
Q7890
STOP

v39=TAPE

n3=0

v40=TAPE19
n5=19
v0=n5
2)n4=0

v0=n5

1)v800=0

XPv(100+n4+n3)=LOGv(40+n4+n3)

n4=n4+1
>1,n4≠n5
v90=0
v89=0
v88=0
v87=0
v92=0

n8=0

4)v1=0
v2=0
v3=0
v4=0
v5=0
v6=0
v8=0
n4=0
```

```
3)v800=0
XPv(400+n4)=EXP(v39Xv(100+n4+n3))

SPv(240+n4)=v(100+n4+n3)Xv(400+n4)

v87=v87+v(400+n4)
v88=v88+v(240+n4)
v89=v89+(v(400+n4)Xv(400+n4))
v90=v90+(v(240+n4)Xv(240+n4))
v92=v92+(v(400+n4)Xv(240+n4))

n4=n4+1
→3,n4≠n5

v91=v89-(v87Xv87)/v0
v93=v92-(v87Xv88)/v0
v94=v90-(v88Xv88)/v0

→11,n3≠0
n4=0
12)v95=v(40+n4)
PRINTv95,1105
n4=n4+1
→12,n4≠19
=
 PRINTv800,1105
n4=0
13)v96=v(100+n4+n3)
PRINTv96,1105
n4=n4+1
→13,n4≠19

PRINTv800,1105
n4=0
14)v97=v(400+n4)
PRINTv97,1105
n4=n4+1
→14,n4≠19

PRINTv800,1105
n4=0
15)v98=v(240+n4)
PRINTv98,1105
n4=n4+1
→15,n4≠19
PRINTv800,1105
11)v800=0
```

```
PRINTv87,II05
PRINTv88,II05
PRINTv89,II05
PRINTv90,II05
PRINTv92,II05
PRINTv91,II05
PRINTv93,II05
PRINTv94,II05
PRINTv800,II05
PRINTv800,II05

→9,n3≠0
n3=I
n5=7
→2
9)v800=0
→5,n3=I
→6,n3=2
→7,n3=3
→8,n3=4
→I0,n3=5

5)n5=n5+I
→2,n5≠I2
n5=5
n3=2

6)n5=n5+I
→2,n5≠I2
n5=5
n3=3

7)n5=n5+I
→2,n5≠I2
n5=5
n3=4

8)n5=n5+I
→2,n5≠I2
n5=5
n3=5

I0)n5=n5+I
→2,n5≠I2

(→0)
8
```



FLOW DIAGRAM 2