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**The two types of nucleic acid during mitosis.** By W. JACOBSON and M. WEBB. *Strangeways Research Laboratory, Cambridge, England*

The histological demonstration of the occurrence of deoxyribonucleoprotein (DRNP) in the chromatin network of the resting nucleus and in the chromosomes during cell division by the Feulgen method or staining with methyl green is well known. The occurrence of ribonucleoprotein (RNP) in the nucleolus and in the cytoplasm of immature or actively working cells has also been established by staining these substances either red with pyronine or blue with Leishman, Jenner-Giemsa or Wright's stain.

The following investigation describes the behaviour of ribonucleoprotein during mitosis. The method used was May-Grunwald-Giemsa's or Leishman's stain after fixation in methanol. Smears of normal and leukaemic bone marrow cells, fibroblasts and osteoblasts grown *in vitro*, and the intestinal mucous membrane of the mouse were investigated. The method was shown to stain RNP dark blue and DRNP red-purple (Table 1). This was demonstrated by

TABLE 1. Distribution of nucleoproteins in cells during the intermitotic period

| | Deoxyribonucleoproteins (purple-red) | Ribonucleoproteins (blue) |
|---|---|---|
| Nucleus: Chromatin | + | – |
| Nucleolus | – | + |
| Cytoplasm | – | + |

+ = present. – = absent.

*in vitro* experiments with the isolated nucleoproteins and by incubating the tissues with enzymes which digest specifically the one or the other nucleoprotein. Thus after treatment of the cells with ribonuclease all blue-staining material was removed, whereas digestion with deoxyribonuclease removed the red-purple staining components. The complete inhibition of proteolytic enzymes, present as contaminants in the deoxyribonuclease preparation, was achieved by the addition of cysteine or hydroxylamine. Thus the observed effects were due specifically to the digestion of deoxyribonucleoprotein.

During the prophase of cell division (Table 2) the chromosome threads contain DRNP but not RNP, and they stain red-purple. The nucleolus contains RNP but not DRNP and stains dark blue.

TABLE 2. Distribution of nucleoproteins in cells during mitotic division

| | Deoxyribonucleoproteins (purple-red) | Ribonucleoproteins (blue) |
|---|---|---|
| Prophase chromosomes | + | − |
| Metaphase chromosomes | + | + |
| Anaphase chromosomes | + | + |
| Telophase chromosomes | + | − |
| Cytoplasm (particularly area between anaphase chromosomes) | − | + |

+ = present. − = absent.

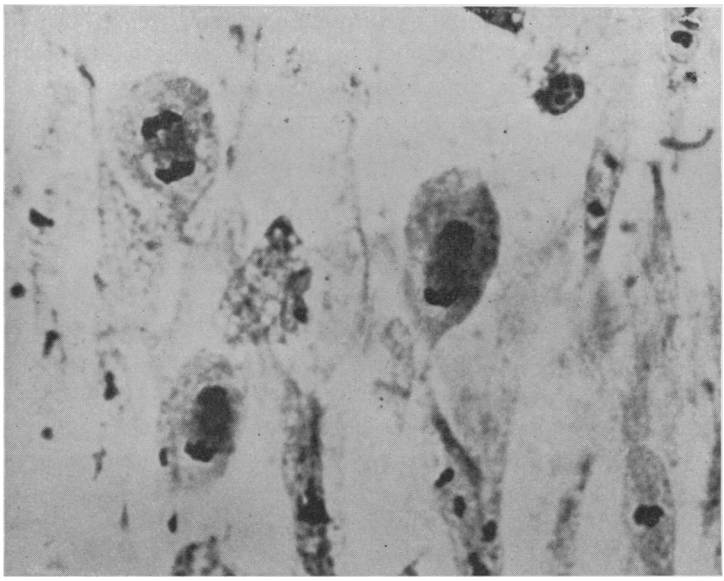

Fig. 1. Three osteoblasts in anaphase: chromosomes dark blue-black, cytoplasm pale blue, area of cytoplasm between the two sets of chromosomes in each of the three dividing cells bright blue, due to its high content of ribonucleoprotein. Methanol, May-Grunwald-Giemsa. ×1200.

During metaphase, after the nucleolus and the nuclear membrane have disappeared, the chromosomes contain *both* types of nucleoproteins, and they stain dark blue-black. In anaphase when the two sets of chromosomes move apart, the chromosomes still stain dark blue-black as in metaphase (Table 2). When the cells are treated with ribonuclease the blue-staining ribonucleoprotein is removed from the metaphase and anaphase chromosomes. They now stain red-purple due to their residual deoxyribonucleoprotein. Conversely, after treatment with deoxyribonuclease, in the presence of proteolytic inhibitors, the purple-red staining deoxyribonucleoproteins of the meta- and anaphase chromosomes are removed and both meta- and anaphase chromosomes now stain blue, due to their residual ribonucleoprotein.

While the two sets of chromosomes move towards the cell poles in anaphase, blue-staining ribonucleoprotein comes off the chromosomes. At the same time, ribonucleoprotein can be shown to be present in the cytoplasmic area between the two sets of chromosomes in a concentration higher than in the surrounding cytoplasm (Table 2 and Fig. 1). A 10 min. treatment with ribonuclease will digest away this material.

In telophase the chromosomal threads have lost nearly all blue-staining ribonucleoprotein; they stain red-purple again, as in prophase, due to their deoxyribonucleoprotein content and the nucleolus is being reformed which stains dark blue, due to its ribonucleoprotein content.

The normal functioning of folic acid appears to be essential for the processes occurring during anaphase since folic acid antagonists, which block the function of folic acid, will arrest mitosis in metaphase.

## A circuit giving a continuous volumetric respiratory tracing.

By J. N. Mills. *Department of Physiology, University of Cambridge*

Douglas & Haldane (1912) described a respiratory circuit with which the human subject could breathe, for an indefinite period, air of constant oxygen percentage. Carbon dioxide was absorbed by alkali and oxygen admitted automatically to replace that used in metabolism. If the large bladder in this circuit is enclosed in an airtight bottle connected to a spirometer it is possible, with certain precautions, to obtain a continuous volumetric respiratory tracing.

REFERENCE

Douglas, C. G. & Haldane, J. S. (1912). *J. Physiol.* **44**, 325.

## A method for drawing micro-capillary tubes.

By R. A. Weale. *Vision Research Unit, Medical Research Council, Institute of Ophthalmology, Judd Street, W.C.* 1

The apparatus described below can be set up without difficulty in any laboratory. It consists of a clamp (*C*) holding the glass tube (*T*) which is to be drawn out, a heating-coil (*H*) surrounding the tube, and a weight (*W*), which draws the glass when it is molten (Fig. 1*a*). The framework supporting the set-up is made from Kee-Klamp scaffolding. The glass tube is fixed in *C* by the pressure of a piece of rubber tubing held tightly against the brass block (*B*) by two screws (*S*). Vertically beneath it is the heating coil which consists of two complete turns of oxynickelchrome wire ($R \approx 2$ Ω/in.). The coil is connected through a switch and a rheostat to a 6 V. supply. The diameter of the coil is approximately 4 mm., and that of the tubing 1·5 mm. external and 0·75 mm. internal.

One end of the tube is bent into a hook so as to ensure that the line of pull is collinear with the axis of the tube; the latter is threaded through $H$ and $C$, and the screws are tightened.

The weight $W$ ($\approx$540 g.) is then suspended from the hook, steadied and the current switched on. Care must be taken lest the coil touch the tubing.

The advantages of this apparatus are these: it is easy to make micro-capillaries of about 3 $\mu$ in diameter; smaller capillaries (less than 1 $\mu$) have also been produced. Once the current and the spacing of the heating-turns are fixed a large number of similar capillaries can be obtained; the production of each does not take more than about 2 min. Casualties are due only to negligence; the percentage of micro-capillaries fit for use as micro-electrodes

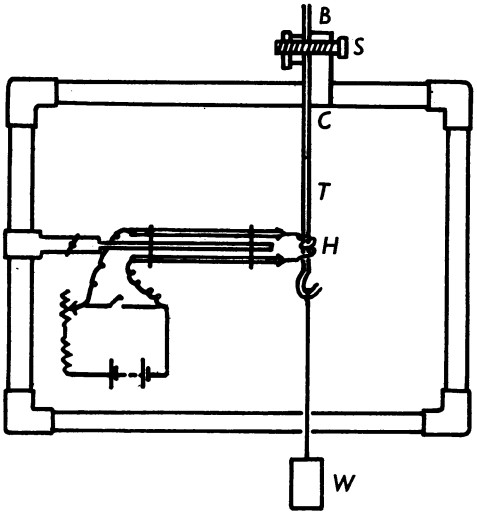


Fig. 1 *a*.

Fig. 1 *b*.

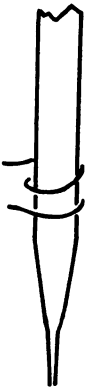

Fig. 1 *c*.

is upward of 75. The shape of the tip can be fixed in a manner which is impossible when a micro-flame is used: a closed spacing of the heating-turns (Fig. 1*b*) produces a tip with a large-angle apex, which terminates in a small elongated cylinder, the actual micro-capillary. When the coil temperature is high (or the weight $W$ large), this cylinder can be drawn out into a 'whisker'. Experience shows that this protrusion is resilient when less than 1 mm. long. A wider spacing of the turns (Fig. 1*c*) gives rise to an entirely different tip; it is conical in shape, sharper and appears to be more brittle. In either case it is found that the glass melts more quickly when it is as near to the coil as possible without actually touching it, i.e. the coil and tube should not be coaxial. This asymmetry does not affect the shape of the tip adversely. Extra heating turns tend to make the tips rather longer.

**Contractions of muscle produced by synchronous and asynchronous motor volleys.** By P. A. MERTON. *Neurological Research Unit of the Medical Research Council, National Hospital, Queen Square, London*

It has been found that if a maximal motor volley is made less synchronous the mechanical twitch of the muscle becomes considerably smaller and briefer, a result which is quite contrary to ordinary expectations. The demonstration is on the human adductor pollicis muscle, tension being recorded by a strain gauge device connected to the proximal phalanx of the thumb. The ulnar nerve is stimulated simultaneously at two points 5 cm. apart near the wrist, with the stimulus distant from the muscle maximal and the nearer stimulus

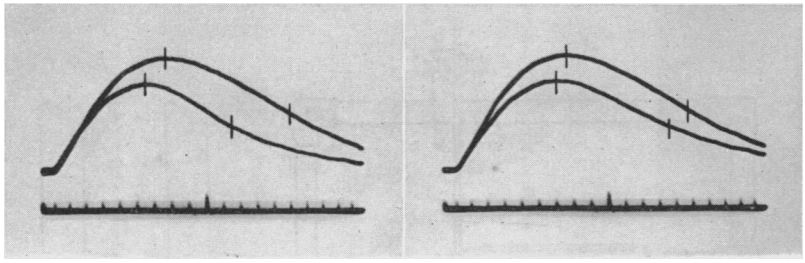

Fig. 1. Approximately isometric twitches of the human adductor pollicis muscle in response to stimulation of the ulnar nerve at two points. Peak and half-relaxation times marked. Time signals 10 msec. and 0·1 sec. Left. Top: maximal shock at top electrode. Bottom: the same with a simultaneous half-maximal shock at lower electrode. Right. Top: as before. Bottom: submaximal shock at top electrode only; twitch same height as left bottom, for comparison of shape.

half maximal. The resulting motor volley involves the same total number of fibres as with a single maximal shock, but it now arrives at the muscle in two half volleys separated by an interval equal to the conduction time between the two electrodes (about 1 msec.). With such an asynchronous volley the twitch tension is reduced as much as 30%, the peak brought forward and the time to half relaxation reduced (Fig. 1). The interval between half volleys can be varied from 0 to 2·5 msec. by sending in the smaller stimulus either before or after the maximal shock. Nevertheless, no further diminution occurs with intervals greater than 0·5 msec. This very brief time in which the effect reaches its maximum is difficult to reconcile with any mechanical explanation of the type put forward by Rushton (1932) for a similar phenomenon in frog muscle. Measurement of the accompanying action potentials shows that their small decrease in height with double stimulation is fully accounted for by the known asynchrony; hence the decrease in twitch tension cannot be due to neuromuscular block, which indeed could not explain the marked changes in twitch shape. The simpler possible explanations seem, therefore, to be inadequate but no alternative analysis of the phenomenon is offered. Identical

results have been obtained on the isolated tibialis anterior muscle of the cat.

Another manifestation of the same effect is seen when the nerve is stimulated at a distance from the muscle; at the elbow instead of the wrist. The longer conduction distance allows the faster travelling nerve impulses to gain a lead and so produces asynchrony in the instants of arrival of impulses at the muscle. As before, this reduces the height and changes the shape of the twitch. If the shock at the elbow is followed by one at the wrist timed to fall just after the elbow volley has passed, the wrist shock is quite ineffective, however strong. So exactly the same motor fibres are involved in the two cases. If the wrist shock just anticipates the elbow volley, the volley becomes synchronous and the twitch assumes the same form as that from the wrist shock alone. In this experiment the shock at the elbow causes contraction of the ulnar muscles of the forearm which jerks the hand and makes a small mechanical contribution to the record. This is a complicating factor, but the experiment can be arranged so that it is kept constant.

REFERENCE

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**Normal and cirrhotic livers in Africans and Europeans.** By H. LEHMANN. *Pathological Laboratory, Tunbridge Wells District Hospital, Pembury, Kent*

The conversion within a few months of a cirrhotic liver into an almost normal organ is demonstrated in serial biopsies performed on African subjects. (See also 'Recovery of a fatty liver as demonstrated by sinal biopy' (Lehmann & Hutton, 1950).) The results are comparable to those obtained on rats by Sellers, Lucas & Best (1948) and on microscopical examination special stains are required to demonstrate any abnormal deposition of fibrous tissue. But in contrast to the work of the Canadian authors the emphasis in the cure was on treatment of infections and infestations rather than on special dietary measures.

It has been occasionally asserted that all Africans suffer from liver cirrhosis in various degrees. Liver biopsies are shown from Africans—ill and healthy—which are as normal as biopsy or post-mortem specimens from normal Europeans.

Occasional invasion of portal tracts by round cells can be demonstrated in all adult livers—even in post-mortem specimens from healthy Europeans who died from accidents.

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**Variations in the lipid in the kidney of the cat correlated with sexual activity.** By MARY C. LOBBAN*. *The Physiological Laboratory, University of Cambridge*

Interest has recently been renewed in the peculiar intracellular lipids found in the kidney of the cat (Smith, 1920; Modell, 1933; Gairns & Morrison, 1949) and other carnivores (MacNider, 1945; Hewer, Matthews & Malkin, 1948).

It has been suggested that the variations in kidney lipids might be correlated with the state of activity of the gonads. With this idea in view, the kidneys of some forty normal cats have been examined, together with those of cats in which the state of sexual activity has been influenced by experimental procedures. The extent of the fat deposits in the kidney of the adult cat can be correlated with sexual activity, most fat being present in the kidney of the pregnant female and the castrate male, while that of the anoestrous female contains no fat, or very little.

The cytoplasm of the fat-containing cells, which are found mainly in the convoluted portions of the nephron, shows strongly acidophilic and siderophilic tendencies when stained by the Azan and iron-haematoxylin methods.

* With a research grant from the Agricultural Research Council.

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**Steel microelectrodes.** By P. O. BISHOP and R. COLLIN. *Department of Anatomy, University College, London, and Department of Human Anatomy, Oxford*

A simple method for the preparation of fine steel needles with a tip diameter as small as $2\mu$. is of interest to those using certain methods of microdissection, while the production of microelectrodes of these dimensions has possible applications in leading off focal potentials from muscle and from peripheral and central nervous tissue. A technique for making fine steel microelectrodes without the laborious grinding usually involved is described together with their insulation and testing.

While using the iron deposition method of Marshall (1940) for histological localization of microelectrodes, it was noticed that the very small current involved removed a relatively large portion of the fine tip in a few seconds. Use was made of this observation in the following manner. Ordinary steel

sewing needles with a small diameter (size 9–12) were fitted in a pin vice connected to the positive pole of a 2 V. accumulator. The negative pole was connected to a stout piece of copper wire placed just under the surface of a molar solution of hydrochloric acid in a beaker. When the needle is moved in and out of the solution the metal is dissolved away by electrolytic action. As the tip is exposed longest to this action and the shank least a very gradual and even taper is produced. To ensure an even surface it is of prime importance that the products of reaction should be removed from the needle by washing in running water and wiping between two layers of cloth or against the edge of a piece of filter paper. In a few minutes it is possible to produce a steel needle with a taper that may extend up to 2 cm. or more and have a final tip diameter as small as $2\mu$. (cf. Fig. 1). When coated with insulating material such a microelectrode penetrates nervous tissue very readily, causes minimal damage and is surprisingly robust. Stainless steel wire (Johnson, Matthey and Co. Staybrite steel wire 37 s.w.g.) may be treated in a similar manner but in this case a 4 V. accumulator is required.

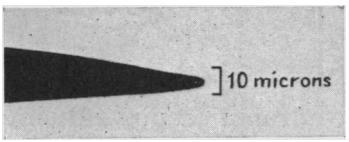


**Fig. 1. Microphotograph (retouched) of the tip of a steel needle prepared by the method described.**

Before applying the insulating coat the needles were thoroughly degreased by washing in alcohol, then ether, followed by dipping in a commercial soapless cleanser and washing off the excess with ether. The most suitable insulating material of several tested was Bakelite L3128. The needles were dipped in the varnish, the surplus removed by means of a camel hair brush to prevent the formation of 'blobs' and the solvent evaporated off by placing the needles in an oven at 130° C. for 5 or 10 min. This is repeated once or more according to the amount of bare tip required after which the needle is baked for at least half an hour at 150° C.

The needles were next tested for uniformity of insulation. The simple test described first proved quite efficient when tested by the more exacting method. The needle is connected to one side of a sine wave generator oscillating at 500–1000 c.p.s. The other side of the oscillator is connected in series with headphones to a very fine smooth wire which is run over the surface of the needle. Even fine pinholes demonstrate their presence as a click heard in the phones. The auditory method of testing allows the procedure to be observed under a binocular microscope. A more thorough method is to use, in place of the smooth wire, a piece of fine cloth stretched taut and well moistened with

saline (0·7%). The needle is inserted through this and a circuit is made through the pinholes by means of the saline contact.

An attempt was made to measure the length of the bare area at the tip of the needle by inserting it by means of a micromanipulator through a piece of gold leaf mounted on a frame. Contact was made and broken within a space of 10$\mu$., the smallest distance measurable with the micrometer screw.

When tested by means of an a.c. bridge and through saline (0·7%) these electrodes gave an impedance ranging from 5 to 100 k$\Omega$ measured at 1000 cyc./sec. A potential of under 5 V. was found necessary, otherwise the insulation was broken down.

The resistance of the insulating layer measured by a 1·5 V. cell and a sensitive galvanometer was above 200 M$\Omega$. There is thus a high safety factor when dealing with biological material.

Thanks are due to Mr David Causley for his help in the development of the technique.

REFERENCE

Marshall, W. H. (1940). *Stain Tech.* **15**, 133.

## Hormones in the adrenal venous effluent.

By I. E. Bush*.
*The Physiological Laboratory, University of Cambridge*

By means of parallel paper chromatograms adrenal cortical extracts, steroid extracts of dog adrenal venous blood and pure steroids have been compared. Free steroids are first separated off by partition with 80% isopropanol. Individual steroids are then separated by acetylation and chromatography on alumina-impregnated paper.

Glandular extracts yield a group of compounds at $R_F$ 0·7 and $R_F$ 0·95. The known active corticoids are all at $R_F$ 0·95.

Over a period of 6 hr. one adrenal gland secreted none of the known corticoids but only the unknown compounds at $R_F$ 0·7. These have been partially split into three components corresponding to the glandular compounds.

In another gland-effluent 5 hr. collection yielded the unknown compounds but only one known corticoid. This was identified as 17-hydroxy-corticosterone.

Rough estimation by the colour of an iodine reaction indicated a rate of secretion of several mg./hr.

* Work undertaken during the tenure of an M.R.C. Studentship.

**Demonstration of nerve endings in the rat diaphragm.** By C. SITARAMAYYA*. *Department of Physiology, King's College, University of London*

In an investigation of the nerve endings present in the rat diaphragm the following were noted:

(1) The presence of typical motor nerve endings.

(2) The localization of these endings to a region approximately 2 mm. peripheral to the entry of the phrenic nerve.

(3) The apparent absence of such endings in other regions of the diaphragm.

(4) The region where the endings are seen corresponds with that in which motor end plate potentials are maximally obtained by Knox and others.

Various techniques have been employed—the most satisfactory being that of Gairns.

* Madras Government Deputationist.

REFERENCE

Hajdu, S. & Knox, J. A. C. (1950). *J. Physiol.* **111**, 43.

**The movements of the epiglottis during deglutition (with film).** By F. H. KEMP. *The Nuffield Institute for Medical Research and The Institute of Social Medicine, Oxford*

There is still controversy as to the behaviour of the epiglottis during swallowing. The popular theory that it folds down over the entrance to the larynx to prevent food entering the airway has been disproved. It has been shown that there are several means of protecting the airway, of which the most important is the contraction of the glottis, and that the tongue of the epiglottis can be entirely removed without any apparent ill effects (Magendie, 1823). Stuart & M'Cormick (1892) observed a patient with a lateral pharyngostomy stoma, and noted that when he swallowed the epiglottis remained upright. Unfortunately, their account is not complete. Various attempts have been made to analyse the movements of swallowing by radiography and with the aid of the fluorescent screen, but this presents difficulties owing to the action taking place too quickly. Barclay (1933) attempted serial radiography, but technical limitations prevented him from obtaining satisfactory records of all phases of the motion. He observed that as the bolus went down, the epiglottis was first pushed back against the posterior pharyngeal wall and subsequently retracted forwards so that it projected 'like a rock under a water fall'. He was unable to see what happened after the bolus passed, and his conclusions were that the epiglottis did not seem to take any vital part in the act of swallowing, or in closing off the pharynx. Negus (1929), who studied the problem clinically and dissected many species, concluded that the epiglottis serves very little useful

purpose, being part of an apparatus which in certain lower animals is used in olfaction.

Thirty normal men and women have been examined with apparatus devised by Ardran & Tuckey, whereby serial radiographs were obtained at the rate of 25 frames/sec. These films show that when the apex of the bolus reaches the vallecula it displaces the epiglottis backwards against the posterior pharyngeal wall. Then, as the main mass of the bolus enters the pharynx, the larynx is pulled upwards and forwards and the epiglottis is drawn forwards. The lumen of the larynx is constricted and arched backwards but is not covered by the epiglottis. In the concluding phases of swallowing, as the bolus is squeezed out of the pharynx, the larynx bends backwards and the epiglottis becomes turned down so that it folds tightly over the laryngeal entrance. When re-inflation of the airways takes place the larynx falls back to its normal position, and as it does so the epiglottis sweeps upwards, and if any food has been forced into the laryngeal vestibule it is then driven out into the pharynx. The larynx is re-inflated from below and the pharynx from above.

In order to confirm these findings a silver clip was placed upon the tip of the epiglottis of a volunteer and a further series of films were taken, with the subject swallowing water. From these it is easy to follow the movements of the epiglottis. Several frames show two images of the clip, since the film is stationary during two 50-cycle impulses (at 25 frames/sec.). From this the maximum speed of movement can be calculated; approximately 1 cm. in 20 msec. This explains the difficulty previous observers have encountered in visualizing the movements of the epiglottis.

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Stuart, T. P. A. & M'Cormick, A. (1892). *J. Anat., Lond.*, **26**, 231.

**Rate of gaseous nitrogen elimination on breathing oxygen.** By WALTER M. BOOTHBY and GUNNAR LUNDIN. *Aero Medical Unit, Mayo Foundation, Rochester, Minn., U.S.A., and the Laboratory for Aviation Medicine, Institute of Physiology, University of Lund, Sweden*

Boycott, Damont & Haldane (Admiralty Committee, 1908) for preventing 'bends', successfully utilized the hypothesis that saturation, desaturation with neutral gases is as predictable in humans as in physical solutions, if proper half-time is assumed. Campbell & Hill (1933) demonstrated goat's fat did not follow the half-time corresponding to *in vivo* saturation.

Behnke (U.S. Navy) and associates (1935–41) reported extensive nitrogen elimination studies, one for 15 hr.

Boothby, Lovelace & Benson (for U.S. Air Force, 1940) first showed accumulated tissue nitrogen elimination is a straight line on log-log paper: $N_m = at^b$ (data released first as a semi-log plot).

Boothby, Lundin & Helmolz (1948), using modified instantaneous Lilly-Anderson Nitrogen Meter (Johnson Foundation for U.S. Navy), devised pulmonary efficiency and circulation rate tests.

Nitrogen 'wash-out' elucidates errors in short metabolic tests (oxygen closed circuit). Hyperventilation reduces alveolar $fN_2$ about 1% vitiating assumptions used in calculating alveolar R.Q. in 'near-steady states'.

Data presented on slides.

**Comparison of arm and general body sweat.** By RUTH VAN HEYNINGEN and J. S. WEINER. *M.R.C. Climatic and Working Efficiency Unit, Oxford*

The main osmotic constituents of arm sweat are chloride, lactate and urea (Weiner & van Heyningen, 1949). We have compared sweat collected simultaneously in an arm bag and from the general body surface with respect to these three solutes.

Two subjects were exposed to fourteen different environments. Four cycles of 20 min. step-climbing and 10 min. rest were performed in a large bath. The man and bath were then washed with distilled water and the solutes estimated in the mixture of sweat and washings. Body water loss was calculated from change in body weight, allowing for water drunk and urine passed.

The proportion of chloride to lactate increases with environmental thermal severity, under both dry and humid conditions. While in any exposure the solutes from the two situations constitute very similar mixtures, the relative output of solutes to that of water is such that arm bag sweat is always more concentrated than that from the body.

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Weiner, J. S. & van Heyningen, R. (1949). *Nature, Lond.*, **164**, 351.

**Clot contraction.** By BESSIE M. STILL. *Department of Chemical Pathology, St Mary's Hospital, London*

This work was carried out to investigate a possible association between clot contraction and fibrinolysis and to elucidate further the role of platelets in the contraction process. Clot contraction was determined by the method of Macfarlane (1939), and the number of adhesive platelets according to the technique of Payling Wright (1941).

The experimental results have shown that contraction is not associated with fibrinolytic agents, or with extra-platelet thromboplastin or with thrombin. First, contraction was found to be proportional not only to the number of whole platelets in the plasma but also to their adhesiveness. Secondly, an inverse relationship was observed between the number of adhesive platelets necessary for contraction and the fibrinogen concentration.

These two facts support the platelet theory of contraction and suggest that the action of the platelets as foci for the laying down of fibrin is important as a means of anchoring the intersections and ensuring contraction of the whole mass of the clot, rather than contraction of individual fibres.

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**Haemolysis of foetal sheep erythrocytes in hypotonic saline investigated by a photo-electric method.** By W. F. WIDDAS. *Department of Physiology, St Mary's Hospital Medical School, London*

A photo-electric method has been employed in studying the rate of haemolysis of foetal sheep erythrocytes in hypotonic saline.

Analysis of the results obtained from experiments with erythrocytes from foetuses of 50–120 days foetal age gives a satisfactory fit to theoretical equations advanced by Jacobs (1926).

By using this method of analysis to obtain a measure of the cell permeability to water it is found that there is a progressive increase of permeability with foetal age.

From the detailed results it is also adduced that cells of widely different permeability do not exist in one and the same blood, and the progressive change with foetal age is therefore interpreted as an intravascular change in the properties of the cells.

REFERENCE

Jacobs, M. H. (1926). *Harvey Lect.* **22**, 146.

**Determination of the fat and cellular tissues in living animals.** By R. A. MCCANCE and E. M. WIDDOWSON. *Medical Research Council Department of Experimental Medicine, Cambridge*

The extracellular fluid volume was determined with thiocyanate, and total body water by measuring the volume into which a known weight of urea distributed itself when taken by mouth. The concentration of urea in the

water of the blood was followed for some hours, and by measuring the urea excreted before and after giving the urea the amount of the dose still in the body at various times was calculated. The weight of the cell mass was obtained on the assumption that 67% of it was water. An allowance was made for minerals, and the amount of fat in the body was obtained by difference.

The method has so far only been applied to man, but it has possibilities for animals. The results which have been obtained with it are so far mainly of medical interest and are being published elsewhere with details of the method.

**The equilibrium between carbon monoxide and sheep haemoglobin at very high saturations.** By F. J. W. ROUGHTON. *Department of Colloid Science, University of Cambridge*

In this range, the equilibrium between CO and haemoglobin should, on the intermediate compound hypothesis, be given by

$$\frac{y\ (\text{percentage saturation})}{100} = \frac{3+4L_4p_{CO}}{4+4L_4p_{CO}}, \qquad (1)$$

$L_4$ being the equilibrium constant of the reaction $CO + Hb_4(CO)_3 \rightleftharpoons Hb_4(CO)_4$. Equation (1) has been verified by new experiments for $y = 98\%$ to $y = 99{\cdot}7\%$. The value of $L_4$ thence calculated varies considerably with pH and temperature. $K_4$, the corresponding constant for the $O_2 + Hb_4O_6 \rightleftharpoons Hb_4O_8$ reaction, is obtainable indirectly by dividing $L_4$ by $M$, the partition constant of CO and $O_2$ for haemoglobin. $K_1$, the equilibrium constant of the $O_2 + Hb_4 \rightleftharpoons Hb_4O_2$ reaction, is determined directly (Paul & Roughton, 1949), and with two of the intermediary constants known, a more decisive application of the theory than heretofore to the oxyhaemoglobin dissociation becomes possible, with fresh light on the physical chemistry of the $O_2$-Hb reaction.

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**Inherent acclimatization of indigenous West Africans.** By W. S. S. LADELL. *Colonial Medical Research Committee's Laboratory for Hot Climate Physiology, Oshodi, near Lagos, Nigeria*

The inherent acclimatization to severe heat and humidity of a group including all main Nigerian tribes was equivalent to that acquired by non-acclimatized Europeans in England from 3 days' artificial acclimatization. Artificial acclimatization of Nigerians improved their performance in the heat, but their rate of improvement fell off earlier than it had done with Europeans

being acclimatized in London. The Africans reached in 6 days the same total sweat production per exposure that the Europeans had taken 10 days to reach; in both cases sweating was not increased markedly with further exposures. Sweat per unit surface area was, however, greater for 'near-fully acclimatized' Africans than it had been for 'near-fully acclimatized' Europeans. Sweat-gland fatigue developed earlier in Africans than in Europeans, total cessation of visible sweat sometimes occurring within 90 min. The chloride content of sweat from Africans was significantly less than that of sweat from Europeans.

**Adenosine triphosphate (ATP) and acetylcholine (ACh) in the contraction of striated frog muscle.** By A. B. L. Beznák. *Physiology Department, University of Birmingham*

ATP has no direct ability to contract striated frog muscle (rectus abdominis in Ringer). When following the addition of ATP a contraction develops (Buchthal & Folkow, 1944; Buchthal, Deutsch & Knappeis, 1944) it is due according to our experiments to ACh (or a like substance) appearing in the bath.

Many muscles are ATP refractive (Abdon, 1942).

The 'ATP-contraction' develops slowly, and it is small; eserine potentiates it, or turns a refractive muscle into sensitive; D-tubocurarine or atropine abolish the ATP effect. The ATP bath, in which a rectus has slowly contracted, causes, added to another rectus, a quick ACh-like contraction. Removal of the ATP by Harpur & Quastel's method (1949) (which leaves ACh unaltered) does not diminish the ability of the bath to cause contraction. This remaining activity is potentiated by eserine; and abolished by curarine, atropine and alkali.

Since the ACh content of the ATP bath often becomes greater than that of the rectus, the ACh appears in the bath in consequence of synthesis and not of mere liberation.

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**Some observations on the antidiuretic properties of plasma and serum of rats.** By S. E. Dicker and M. Ginsburg. *Department of Pharmacology, University of Bristol*

Rats' serum injected subcutaneously (1 ml./100 g.) in hydrated rats is antidiuretic, confirming observations of Birnie, Jenkins, Eversole & Gaunt (1949); this antidiuretic activity is approximately of the same magnitude as that pro-

duced by 0·6 milliunit of vasopressin per 100 g. body weight. The activity is not lost after standing at 4° C. for 18 hr. Heparinized rats' plasma did not have a definite antidiuretic activity. Vasopressin added to serum (1·5 mU./ml.) was rapidly inactivated; vasopressin added to plasma (1·5 mU./ml.) was not inactivated. After heat coagulation of serum the supernatant fluid had approximately twice the antidiuretic activity of fresh serum. Plasma or serum from rats in which it is likely that antidiuretic hormone is circulating in the blood (e.g. dehydrated rats and rats injected with large amounts of vasopressin) did not have a greater antidiuretic activity than plasma or serum from normal animals.

These observations suggest that the antidiuretic substance of normal rats' serum is not posterior pituitary hormone.

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**Antidiuretic substance in human urine after smoking.** By N. B. G. Taylor and J. M. Walker. *Department of Pharmacology, University of Oxford*

Burn, Truelove & Burn (1945) showed that cigarette smoking inhibits water diuresis in man, and suggested that the nicotine causes an output of antidiuretic hormone from the posterior lobe of the pituitary.

Fourteen experiments have been done on three normal non-smoking human subjects. Specimens of urine passed before and after the smoking of one cigarette were extracted separately by the method of Noble, Rinderknecht & Williams (1939) and tested for antidiuretic activity on rats by the method of Burn. To obtain enough material for assay, extracts from individual experiments were pooled. Extracts of urine passed after smoking had an antidiuretic action which was abolished by treating with N-NaOH for 1 hr. at room temperature, whereas extracts of urine passed before smoking had no antidiuretic action. In three assays, the antidiuretic activity was estimated as equivalent to 10–20 milliunits pituitary (posterior lobe) extract per 100 ml. urine.

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**Uterine sympathin and noradrenaline.** By MONICA MANN and G. B. WEST. *Pharmacological Laboratory, School of Pharmacy, University of London*

Blood taken from the ovarian vein of a non-pregnant cat under chloralose, during intermittent stimulation of the hypogastric nerve, contains substances with the properties of noradrenaline and adrenaline.

The activities, when tested on the non-pregnant isolated rat uterus, the isolated rectum of the week-old chick, and the chronically denervated nictitating membrane of the cat, correspond to mean values of 0·037 $\mu$g./ml. plasma for noradrenaline, and of 0·005 $\mu$g./ml. plasma for adrenaline. Ligation of the adrenal vessels did not affect the result. In two out of thirteen experiments, noradrenaline was not found. These findings are in agreement with those found by direct recording of uterine movement *in situ* (West, 1949).

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**Antidromic vasodilatation in the rabbit's ear.** By PAMELA HOLTON,* *Physiological Laboratory, Cambridge,* and W. L. M. PERRY, *National Institute for Medical Research, Mill Hill, London, N.W. 7*

Antidromic vasodilatation in the sympathetically denervated rabbit's ear, produced by stimulation of the cut great auricular nerve, has been recorded with a photocell. The current passed by the cell is proportional to the light entering it, and is recorded on a moving paper camera through a circuit including cathode followers and a mirror galvanometer.

This method is sensitive to small changes in the degree of vasodilatation; reproducible vasodilatation, lasting 1–3 min., is regularly obtained in response to single shocks of 1·0 msec. duration. Atropine and neoantergan, in doses which abolish the action of intra-arterial injections of acetylcholine and histamine respectively, do not affect antidromic vasodilatation.

Hellauer & Umrath (1948) claimed that the substance causing antidromic vasodilatation was identical with the central synaptic transmitter of sensory nerves, and that strychnine inhibited the enzymatic destruction of this substance. Strychnine, however, does not affect antidromic vasodilatation.

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**Variations in mammary blood flow during the menstrual cycle.** By V. R. PICKLES. *Department of Physiology, King's College, Newcastle upon Tyne*

Daily measurements by means of an instrument described previously have been made over three menstrual cycles on a normal non-lactating subject. The results suggest that the rate of mammary blood flow is least in the 2nd week and greatest in the 4th week of the cycle, averaging $36 \pm 10\%$ (S.E. of difference of means) more in the latter week. A smoothed curve of the mammary readings bears a general resemblance to that of body temperature, but the correlation between pairs of individual readings is not significant.

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**Inhibitors of autonomic ganglia on the isolated guinea-pig's ileum preparation.** By W. FELDBERG. *National Institute for Medical Research, Mill Hill, London, N.W. 7*

In order to establish how far stimulation of the myenteric plexus contributes to intestinal contractions produced either spontaneously or by drugs, we require substances which do not reduce the excitability of the muscle fibres as well. Paralysing doses of nicotine do so to a great extent (Emmelin & Feldberg, 1947). Because of a weak atropine-like action D-tubocurarine is also unsuitable, and even tetraethylammonium iodide apparently is not specific. Hexamethonium proved suitable. Paton & Zaimis (1949) discovered its strong paralysing effect on autonomic ganglia.

On the ileum, hexamethonium does not reduce the response to histamine or pilocarpine, that to acetylcholine and potassium very slightly, but that to barium strongly. The barium contractions are, therefore, as suggested by Ambache (1946), partly ganglionic in origin. A piece of ileum close to caecum often exhibits spontaneously quick, sharp contractions, which are abolished by hexamethonium, and thus ganglionic in origin.

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**Variation in the adrenaline motor response of the isolated terminal ileum of the guinea-pig with body weight and exposure to anticholinesterase drugs and atropine.** By A. F. MUNRO. *Department of Physiology, King's College, University of London*

An isolated segment from the terminal ileum of the guinea-pig, unlike that of the rabbit and rat, is contracted by adrenaline, but the strength of the response varies considerably between animals (Munro, 1951). Analysis now shows an inverse relation between body weight and strength of contraction to adrenaline. Foetal gut is, however, relaxed by adrenaline.

The adrenaline response is prevented by ergotoxine and nicotine, but not by TEA. Physostigmine, neostigmine, DFP and TEPP reduce, abolish or reverse the motor response to adrenaline; but the latter inhibits the spontaneous rhythmicity induced by these drugs.

Atropine potentiates the adrenaline contraction and abolishes the inhibitory effect of the anticholinesterases. It also reverses the normal relaxation response observed in the foetus and, occasionally, in very large adult animals.

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**The effect of some anticholinesterase drugs on the isolated tracheal muscle of the guinea-pig.** By W. W. DOUGLAS

The role of broncho-constriction in anticholinesterase poisoning has prompted study of the effect of such drugs on respiratory tract muscle. Eserine sulphate (1 : 10,000,000), TEPP (1 : 10,000,000) and DFP (1 : 1,000,000) each causes the tracheal muscle of the guinea-pig to contract, besides sensitizing it to acetylcholine. The contractions differ from those produced by added acetylcholine, being slower in onset and rate. In this respect they resemble the responses of the gut to cholinesterase-inhibiting drugs (Adrian, Feldberg & Kilby, 1947), suggesting as their cause endogenous formation of acetylcholine. The contraction caused by a maximal concentration of any of these three drugs is less than by a maximal concentration of acetylcholine. TEPP is usually without further constricting effect after maximal doses of eserine. Atropine sulphate (1 : 100,000,000) abolishes the contractions. Washing removes the spasm due to eserine, or high concentration of TEPP (1 : 20,000 for 60 min.). The effect of DFP, however, is more persistent.

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