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Apparatus for the study of (*a*) the absolute threshold of human vision, (*b*) spatial and temporal summation in the retina. By E. J. DENTON and M. H. PIRENNE

Class demonstration of the fundamental laws of colour-mixing in trichromatic and dichromatic vision. By E. J. DENTON and M. H. PIRENNE

Simple class demonstration of chromatic aberration in the human eye. By M. H. PIRENNE

A modified Marey heart lever for class experiments upon *Rana temporaria*. By E. J. DENTON

Method for studying chemical excitants of cutaneous pain in man. By DESIRÉE ARMSTRONG, R. M. L. DRY, C. A. KEELE and J. W. MARKHAM. *Department of Pharmacology, Middlesex Hospital Medical School, London, W.* 1

During the course of human studies on the production of cutaneous pain by various chemical agents we have found that injection techniques, such as intradermal injection, or pricking through a drop of solution, sometimes give unreliable results.

We describe here an alternative method which is very simple, sensitive and satisfactory. A small blister (area rather less than 1 cm.^{2}) is raised on the skin of the forearm by application for 4–6 hr. of a cantharidin plaster (0·2% cantharidin). The epidermis which has separated from the dermis is removed and the exposed blister base is then available for testing. The solutions containing the pain-producing substances (volume about 0·2 ml.) are then applied

to the area at intervals of 5–10 min., and between applications the area is bathed in a solution of the following composition: NaCl, 0·92 g.; KCl, 0·04 g.; $CaCl_2$, 0·024 g.; $NaHCO_3$, 0·015 g.; water to 100 ml.

The advantages of this procedure may be summarized thus:

(1) The pain-producing solutions gain rapid access to the exposed nerve-endings and networks in the superficial layers of the dermis, without any prior pain due to insertion of a needle.

(2) There is no spontaneous pain except during the first 10–15 min. after removal of the epidermis.

(3) Each solution acts upon the same set of nerve terminals, and with suitable intervals between applications the pain receptors are in a comparable state of sensitivity for each test. Fluctuations in sensitivity can be detected by application of standard solutions of KCl or acetylcholine.

(4) The exposed area remains sensitive for up to 2 days, and during this time 50–60 applications have often been made without complications due to fatigue, etc.

(5) Bacteriological sterility of the applied solutions does not appear to be essential. We have seen no infections in some thirty experiments in four subjects.

The recording of pain is simply done by the use of a subjective pain scale. A pressure bulb connected to a mercury manometer and vertical-writing lever allows the subject to record his pain sensations in the form of a tracing on a moving smoked drum. To avoid bias in the making of such records the apparatus is arranged so that the subject cannot see the shape of the tracing which he is making. The constancy of the responses is tested by the application of solutions whose composition and concentration are unknown to the subject. The isotonic washing solution does not cause pain.

In any one person the pain response to a given chemical agent is quite constant in occurrence and character, and the recorded intensity of pain is proportional to the concentration of the pain-producing substance. In these circumstances a 'threshold' concentration can readily be determined. When different chemical substances are applied it is often possible to distinguish differences in the character of the pain which they evoke; for example, it is easy to differentiate the pain produced by acetylcholine from that produced by a hypotonic or acid solution.

The following are some substances and preparations which have been found to produce pain:

Acetylcholine chloride, in concentrations down to 3×10^{-5}.

KCl, in concentrations down to 0·12%.

Histamine dihydrochloride, in two of four subjects, in a concentration of 10^{-3} (as base); below this, usually only itching was recorded.

Hypotonic solutions, e.g. 0·3% NaCl.

Hypertonic solutions, e.g. 3% NaCl.

Acid solutions, but only when the pH falls to about 2·5.

Skin extracts (rat, human).

All these solutions, except those deliberately made hypo- or hypertonic, were made up to be as near isotonic as possible.

Fluid electrodes with a rubber diaphragm. By R. C. GARRY and MARY WISHART. *Institute of Physiology, University of Glasgow*

In order that the strength of a stimulus may be proportional to the reading of a potentiometer, it is essential that the resistance of the electrode system should be high compared with that of the potentiometer. With the usual type of fluid electrode, the nerve only partially blocks the hole between the two chambers and consequently the surrounding fluid offers a low resistance to the stimulating current (Eccles, 1928; Brown & Garry, 1932; Collison, 1933).

In the present design a rubber diaphragm, cut from thin condom rubber, is interposed between the two chambers. The nerve is tied in the usual fashion. The ligature is threaded through the eye of a fine needle which is then used to puncture and to draw the ligature and nerve through the rubber diaphragm. The diaphragm closes down on the nerve whatever its size, thus reducing the fluid shunt to a minimum.

Using square-wave pulses of 1 msec. duration and at a frequency of 50 cyc./sec. we have consistently had adequate stimulation with voltages ranging from 0·5 to 3. At these voltages we have not seen spread to the surrounding muscles. The rubber diaphragm seems to cause no gross injury; effective stimulation persists for six or more hours.

The in vivo *electrode.* The structure and dimensions are shown in Fig. 1A. The electrode is turned from Perspex rod and consists of two parts which fit into one another. When the rubber diaphragm is in position friction holds the two parts firmly together. Each part has to be made in two separate portions to allow insertion of the circular silver ribbon made by beating out the end of a fine silver wire. The ribbon lies in a recess away from contact with the nerve. A groove cut in one portion allows exit of the shank of the silver ribbon when the portions are finally sealed together with chloroform. Close to the electrode the silver wire is soldered to a length of stranded flexible fine copper wire. Polythene tubing of 1 mm. bore is drawn over the lead and inserted into the hole of exit for the silver shank. A watertight joint is made by sealing the polythene tubing in position with chloroform. Before use the silver ribbons are coated with silver chloride.

The ligature attached to the nerve is held in position by a 'Perspex' plug. Filling of the chambers with Ringer fluid or serum is carried out with a hypodermic syringe and blunt bent needle just before insertion of the plug and on

lodgment of the electrode in the animal's body. So that the electrode may be easily grasped by forceps it is useful to file flat the sides of the electrode and the head of the plug. The small size of the electrode allows it to be applied to nerves when only short lengths are accessible.

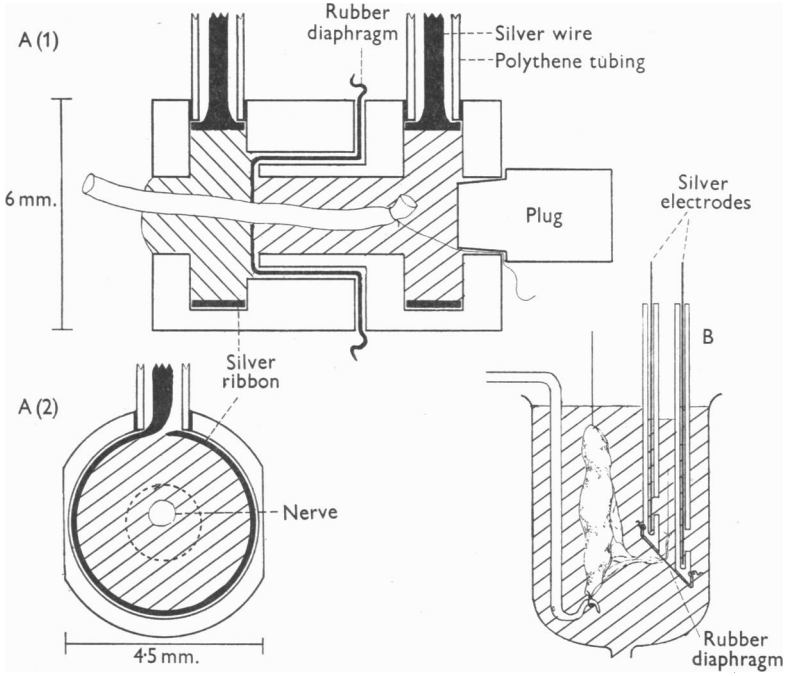

Fig. 1. A(i). Longitudinal section of *in vivo* electrode. A (ii). Transverse section of *in vivo* electrode through recess of chamber. B. Longitudinal section of *in vitro* electrode; not to scale.

The in vitro *electrode.* This design is most useful for stimulation of mesenteric nerves to the gut *in vitro*. This electrode is in one piece and may be bored out of 'Perspex' rod or moulded in acrylic resin (Fig. 1B). Once more the rubber diaphragm ensures that a dense current impinges on the nerve. Voltages of the order of 1–3 continue to give effective inhibition of gut movements over many hours. An electrode of similar design has been used to stimulate the phrenic nerve *in vitro* (Mogey, Trevan & Young, 1949).

We are indebted to Dr W. Malcolm Gibson of our Dental School for moulding the *in vitro* electrode in acrylic resin.

REFERENCES

Brown, G. L. & Garry, R. C. (1932). *J. Physiol.* **75**, 213.
Collison, L. W. (1933). *J. Physiol.* **80**, 14*P*.
Eccles, J. C. (1928). *J. Physiol.* **66**, 118.
Mogey, G. A., Trevan, J. W. & Young, P. A. (1949). *Analyst*, **74**, 577.

Effect of variations in food intake on rats hypophysectomized during pregnancy. By ROSA M. CAMPBELL, I. R. INNES and H. W. KOSTERLITZ

The electrocardiogram of the mouse. By I. R. INNES and H. W. KOSTERLITZ

Modification of the cardio-accelerator effect of electrical stimulation of the sympathetic cardiac nerves by the systemic administration of local anaesthetics. By I. R. INNES and H. W. KOSTERLITZ

The action of adrenaline and noradrenaline on the peristaltic reflex of the isolated guinea-pig's ileum. By VIVIEN PIRIE

Microscopic preparations of nerve endings in the human gum and hard palate. By F. W. GAIRNS

The preparation of Biebl loops and Thiry-Vella fistulae of the ileum of the horse. By F. ALEXANDER. *Rowett Research Institute, Bucksburn, Aberdeenshire*

The principal reason for the lack of knowledge about the digestive processes in the horse has been the surgical problem of making chronic preparations giving access to the intestine. A technique for fistulating the large colon has already been described (Alexander & Donald, 1949). An adaptation of the Biebl loop and Thiry-Vella fistula to the horse ileum has been developed.

The ileum was approached through the flank, the operative site being limited cranially by the 18th rib and caudally by a line parallel to the last rib and immediately in front of the coxal tuberosity.

Anaesthesia was induced with sodium pentobarbital (10 mg./kg.) and maintained by subsequent doses of 2 mg./kg. The last six intercostal nerves were blocked with a 1/1000 nupercaine solution.

In the preparation of a Biebl loop a fold of skin was defined about 5 in. wide and 9 in. long running in the direction of the fibres of the internal oblique muscle. This skin flap was freed from the body wall except at the two ends, and the abdomen entered through an incision in the middle of the exposed fascial surface, cutting across the external oblique and dividing the fibres of the internal oblique and transverse abdominal muscles by blunt dissection. A loop of ileum was brought into the wound, the avascular part of its mesentery incised and the peritoneum and the muscles of the abdominal wall joined with

silk sutures through this incision, leaving a space at each end so that the lumen of the ileum was not occluded. The exteriorized ileum was enclosed within the bipedicled skin flap. The skin of the abdominal wall was closed beneath the loop so formed.

For the preparation of a Thiry-Vella loop the abdomen was approached through the flank, the abdominal muscles being separated by blunt dissection in the direction of their fibres. A loop of ileum, about 50 cm. long and supplied by at least one intestinal artery, was sectioned between clamps. Two stab incisions one above the other were made about 3 in. from the main wound. The ends of the ileal loop were grasped with long clamps passed through the stab wounds and thus drawn through them. It was important to allow at least 2 in. of ileum to protrude from the stab wound before securing with four nylon sutures. Continuity of the ileum was restored by an end-to-end anastomosis using linen sutures. The muscles and peritoneum were re-united with silk and the skin wound with nylon sutures.

I am indebted to Prof. D. M. Douglas for advice on the preparation of Biebl loops.

REFERENCE

Alexander, F. & Donald, D. E. (1949). *J. comp. Path.* **59**, 127.

Some methods of studying gastric motility in sheep and the use of duodenal fistulae and gastric pouches. By M. I. CHALMERS, D. L. DUNCAN and A. T. PHILLIPSON

The role of vitamin B_{12} and other animal protein factors in growth and egg production of birds. By K. J. CARPENTER and J. DUCKWORTH

The effect of different levels of calcium in the diet of the ewe on the degree of resorption of the skeleton during lactation, as shown by radiography. By D. BENZIE, J. DUCKWORTH and R. PEARCY

The effect of varying planes of nutrition during the two years' rearing period, on the growth, health and production of dairy cattle using identical twins as experimental animals. By J. AITKEN and J. CRICHTON

Microbial ecology of the digestive tract. By F. BAKER and M. MASSON

The microscopic study of homogenates of mouse liver in relation to their β-glucuronidase activity. By P. G. WALKER

Bracken poisoning. By G. H. CUSHNIE and M. M. NAFTALIN

Plane of nutrition in relation to early growth of sheep. By J. C. GILL and W. THOMSON

Radiological study of the digestive tract of the ewe. By D. BENZIE

Respiration of the cornea. By M. LANGHAM. *Ophthalmological Research Unit (Medical Research Council), Institute of Ophthalmology, London, W.C.* 1

The apparatus consists of a transparent airtight box of Perspex containing the anaesthetized rabbit, normal respiration being maintained through a tracheal tube connected to the outside atmosphere. Lactic acid was determined by the method of Barker & Summerson (1941). An initial control series for normal rabbits showed a concentration of 96·0 mg.% (wet weight) of lactic acid in the cornea, the ratio of the concentrations in the left and right corneae being $1{\cdot}000 \pm 0{\cdot}019$ (8). With the tank filled with nitrogen the ratio of the concentrations of lactic acid in the nitrogen-exposed cornea (N) to the control (Cont.), i.e. N/Cont. was $1{\cdot}330 \pm 0{\cdot}078$ (5). In contrast, an atmosphere of nearly pure oxygen (O) led to a drop in the concentration of lactic acid, the ratio O/Cont. in this case being $0{\cdot}663 \pm 0{\cdot}078$ (5). These results suggest that the cornea can utilize oxygen obtained directly from the atmosphere and does so under normal conditions. This conclusion is supported by the observation that suturing of the lids together for 24 hr. led to an increase in concentration of lactic acid, i.e. the ratio of concentrations of lactic acid in the experimental and control corneae was $1{\cdot}142 \pm 0{\cdot}049$ (6).

Injection of a bubble of oxygen into the anterior chamber, performed through the sclera so that the cornea remained undamaged, led to a marked decrease in concentration of lactic acid in the cornea (ratio O/Cont. $0{\cdot}703 \pm 0{\cdot}032$ (5)). Injection of a bubble of nitrogen led to an increase in the concentration of lactic acid (ratio N/Cont. $1{\cdot}256 \pm 0{\cdot}049$ (8)). These results indicate that oxygen can diffuse through the layers of the living cornea in both directions. Further, it would appear that in the normal eye oxygen diffuses from the aqueous humour into the cornea where it is utilized.

REFERENCE

Barker, J. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.

The spectral sensitivity of the toad *Xenopus laevis*. By E. J. DENTON and M. H. PIRENNE. *Physiology Department, Marischal College, University of Aberdeen*

Method I. Placed on a black background, *Xenopus* changes its colour from 'white' to 'black' by expanding its skin melanophores when it receives enough light from an opal glass screen overhead. The threshold intensity was found for lights from different parts of the spectrum.

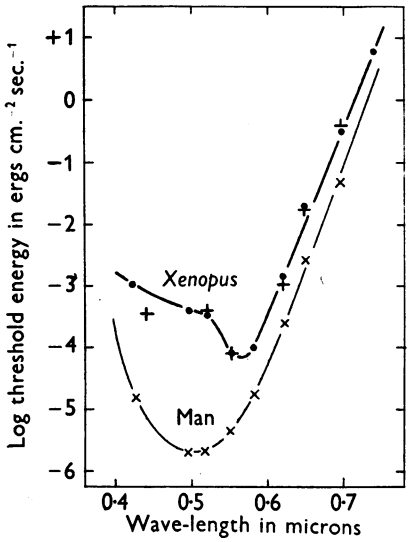

Fig. 1. The absolute measurements give the energy emitted by the extended screen, in ergs/cm.²/sec. + *Xenopus*, method I, mean results. The absolute level is that of the most sensitive animal. ● *Xenopus*, method II, results for one animal. Relative scale, adjusted to the absolute level of method I. A smooth curve is drawn through the points. × *Man*, absolute threshold of one subject measured with the same apparatus. The corresponding smooth curve is the mean scotopic function of Crawford (1949).

Method II. *Xenopus* usually goes into the shade. The threshold value here was the illumination below which an animal failed to go into the shaded part of a tank.

The two methods give curves which agree closely in their shape (Fig. 1). There are individual variations in the absolute thresholds and in the shape of the curve in the blue.

Effect of temperature. Using Method II, one *Xenopus* was found to be $4{\cdot}62 \pm 0{\cdot}09$ (S.D.) log units more sensitive to light of 550 mμ. than to light of 740 mμ. at 9° C., and $4{\cdot}07 \pm 0{\cdot}06$ log units more sensitive at 29° C. The animal was therefore about 3 times more sensitive to red light of 740 mμ. (relative to light of 550 mμ.) at 29° C. than at 9° C.

REFERENCES

Broda, E. E. & Goodeve, C. F. (1941). *Proc. Roy. Soc.* A, **179**, 151.
Crawford, B. H. (1949). *Proc. phys. Soc. Lond.* B, **62**, 321.
Hogben, L. & Slome, D. (1931). *Proc. Roy. Soc.* B, **108**, 10.
Hogben, L. & Slome, D. (1936). *Proc. Roy. Soc.* B, **120**, 158.
Stiles, W. S. (1948). *Transactions of the Optical Convention of the Worshipful Company of Spectacle Makers*, p. 97. London.

The significance of beading in corneal nerve fibres.

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The corneal nerves, stained vitally with methylene blue, exhibit a characteristic beaded appearance. Such beading, however, we have never seen with the biomicroscope. The 'neurofibromata' described by Koeppe (1919) are almost certainly Schwann cells. They are much too large to be beads.

We have never seen beads in really fresh phase contrast slices. We have, however, found them in abundance under phase conditions 2 hr. after the slice was taken. In vitally stained preparations, moreover, we have observed beads arise on nerves originally non-beaded. This is also true of the free intra-epithelial endings.

Last year we described (Rodger, 1950) the presence of non-beaded fibres in addition to the more common delicate beaded ones. At the time we suggested the morphological difference *might* presuppose a difference in function. Destruction of the Gasserian ganglion, however, by Zander & Weddell (1951) has led to total degeneration of the corneal nerves; and we have recently performed in cats extradural ophthalmic neurectomy above and below the cavernous sinus with the same result. Further, in intradural oculomotor neurectomy (contrary to the findings of Nageotte & Guyon, 1938), and in cervical sympathectomy, we found no demonstrable lesion in the cornea. It appears probable, then, that the corneal nerves are all of the same type. Another explanation is required.

Contrary to the usual opinion, not all of the myelin sheathing is lost at the periphery. By the polarizing microscope, the sheaths of some of the nerves may be seen to pass to the apex. Perhaps, therefore, the coarse non-beaded fibres described by us are myelinated ones, and are non-beaded because the myelin to some degree protects the nerve fibres.

There seems every reason to conclude that beading is but evidence of the response of a nerve fibre to an environmental change, physico-chemical or irritant in nature. In support of this, traumatic fragmentation of the corneal fibres is preceded by gross beading.

REFERENCES

Koeppe, L. (1919). *v. Graefes Arch. Ophthal.* **99**, 1.
Nageotte, J. & Guyon, L. (1938). *C.R. Ass. Anat.* **33**, 369.
Rodger, F. C. (1950). *Brit. J. Ophthal.* **33**, 107.
Zander, E. & Weddell, G. (1951). *Brit. J. Ophthal.* **35**, 61.

The minimum number of quanta necessary for vision. By E. J. DENTON and M. H. PIRENNE. *Physiology Department, Marischal College, University of Aberdeen*

According to the quantum theory, there must be near the absolute threshold an intensity range in which physical fluctuations cause uncertainty of seeing. If there are biological variations or experimental errors, this irreducible uncertainty range will generally be widened. Accordingly, the frequency-of-seeing curves published by Hecht, Shlaer and Pirenne were obtained using very special precautions in the hope of eliminating experimental variations as completely as possible. These 'good' curves all have an uncertainty range of about 1 log unit.

Curves with a smaller range were never obtained in these experiments, but 'bad' curves, with a markedly larger range, were. These 'bad' curves were not published because it seemed obvious that they were vitiated by experimental variations. The first curves given by inexperienced subjects—who later gave steeper curves—were often 'bad' curves, for instance. This suggested that the 'good' curves themselves, in comparison with theoretical curves assuming constant organism sensitivity, might be too shallow, but not too steep.

The first theoretical calculations assumed implicitly that the small retinal region covered by the light stimulus contained only one independent light-detector. In such a case, the shape of the curve will depend only upon n, the minimum number of quanta which must be absorbed by the retina to reach the threshold of vision. (We assume that there are no experimental variations.) If several independent light-detectors are involved, however, the theory must be modified. The resulting curve will be steeper than that of the simple theory and will therefore correspond to an apparent n value which is too high.

In spite of these complications, conclusions can be drawn concerning the value of n. For instance, the shape of the curve for various numbers N of independent light-detectors can be calculated assuming $n=2$. The curves become steeper when N is larger, but it is found that, even if N is equal to the total number (about 500) of rods and cones involved in the experiment, the curve remains shallower than the 'good' experimental curves. The hypothesis $n=2$ therefore seems incompatible with observation. Other considerations suggest that n is equal to at least 4. The experiments which lead some authors to suggest that $n=2$ can be interpreted also on the basis $n \geqslant 4$ quanta.

Further observations on the function of the erectores spinae muscles during flexion of the trunk. By W. F. FLOYD and P. H. S. SILVER

Rapid recovery of sweating in the arm after arterial occlusion.

By W. S. S. LADELL. *Colonial Medical Research Committee's Laboratory for Hot Climate Physiology, Oshodi, near Lagos, Nigeria*

In thirty-three experiments on six subjects, the effect of arterial occlusion on the volume and chloride content of lower-arm sweat was followed. Subjects exercised, three in an effective temperature (E.T.) of 27·8° C. and three in E.T. 30° C., at a mean metabolic rate of 170 kg. cal./m.^{2}/hr.; overall sweat rates were 14 g./min. and over. Impermeable bags were worn on both arms with uninflated pneumatic tourniquets bound just above the elbows; lower-arm sweat only collected below the tourniquets. After two 15 min. collecting periods the tourniquet on one side was rapidly inflated to 200 mm. Hg and left inflated for two collecting periods (30 min.). Collecting continued for two further periods after the pressure was released. Tests were carried out on both right and left arms. The most satisfactory comparison was found to be that between the occluded, 'treated', and unoccluded sides in each separate test.

Before occlusion sweat output and chloride content were the same on both sides. In the first 15 min. of occlusion the mean output from the treated arm was 39·2% ± 1·07 (S.E.) of that of the other arm; in the next 15 min. it was 13·0% ± 0·98 (S.E.). Sweating increased immediately the tourniquet was released, and was at the same rate as in the untreated arm in the second 15 min. after restoration of the blood supply. The sweat rate of the untreated arm was not affected by the occlusion of the opposite side; nor was there any depression after the release of the occlusion as described by Randall, Deering & Dougherty (1948).

The chloride content of the sweat from the treated arm was less than that of the opposite arm in the two periods during occlusion, and in the first post-occlusion period, by 9·4, 17·6 and 9·2% respectively. The mean differences in g. NaCl/100 ml. were 0·02, 0·04 and 0·03 ± 0·004, 0·005 and 0·004 (S.E.'s) respectively. These differences are significant by the t test, with P less than 0·001. In the last 15 min. period the chloride content had also risen to that of the opposite side.

This rapid recovery suggests that the rise in chloride content of the sweat during prolonged heavy sweating occurs independently of the amount of activity undergone by any given gland and may possibly indicate that the control of both sweat rate and sweat composition is more central than has sometimes been supposed (Johnson, Pitts & Consolazio, 1944; Robinson, Gerking, Turrell & Kincaid, 1950).

REFERENCES

Johnson, R. E., Pitts, G. C. & Consolazio, F. C. (1944). *Amer. J. Physiol.* **141**, 575.
Randall, W. C., Deering, R. & Dougherty, I. (1948). *J. appl. Physiol.* **1**, 53.
Robinson, S., Gerking, S. D., Turrell, E. S. & Kincaid, R. K. (1950). *J. appl. Physiol.* **2**, 654.

The effect of eserine on the output of acetylcholine from the cat's superior cervical ganglion. By W. L. M. PERRY

Nerve endings in the human gum and hard palate. By F. W. GAIRNS.
Institute of Physiology, University of Glasgow

Recently, Gairns & Aitchison (1950) described the types of nerve endings in the human gum. This work has now been extended to include the endings in the human hard palate. As before, the staining method used was the Bielschowsky-Gros silver technique. Nerve endings are very numerous in the gum and palate; typical corpuscles of Meissner and of Krause lie below the stratified squamous epithelium in their usual sites. Free nerve endings, as in the skin, penetrate the basement membrane to enter the basal layers of the epithelium. Such free nerve endings are more abundant in the palate than in skin or gum. So far Pacinian corpuscles have not been seen in palate or gum, although they may exist in the deepest parts of the muco-periosteum which have not so far been examined.

The most common ending in gum and palate, however, is quite distinct from any known receptor in the skin. These may be large compact or loose unencapsulated 'whorls' of varying shape and size. Many possess one or two terminal filaments which penetrate the basement membrane and may extend to and enter the most superficial layers of the epithelium. Some of these fine filaments end in a tiny knob. A double innervation of the ending as a whole is not infrequently seen. One or more fine non-myelinated strands accompany the large myelinated fibre and become intimately associated with the actual ending.

Both in the gum and palate there is a profuse sympathetic ground plexus similar to that described by Cathcart, Gairns & Garven (1948) in the skin of the human nipple.

REFERENCES

Cathcart, E. P., Gairns, F. W. & Garven, H. S. D. (1948). *Trans. Roy. Soc. Edinb.* **61**, 699.
Gairns, F. W. & Aitchison, J. (1950). *Dent. Rec.* **70**, 180.

The effect of hexamethonium on carotid body responses in the cat. By W. W. DOUGLAS. *From the National Institute for Medical Research, Mill Hill, N.W. 7*

It has been suggested that the afferent pathways from the chemosensory cells of the carotid body may be interrupted by synapses similar to those occurring in the autonomic nervous outflow (Euler, Liljestrand & Zotterman, 1939). If this were the case the effect of stimulation of these cells should be prevented by hexamethonium which exerts a powerful blocking action on autonomic ganglia.

It has been found, however, that the carotid body response to oxygen lack or to cyanide—both stimuli considered to act on the chemosensory glomus cells—was unaffected, or only slightly depressed, by hexamethonium in amounts causing autonomic blockade.

One fact which suggested synaptic transmission of carotid body responses was that typical chemosensory reflexes can be elicited from the carotid body by substances with known ganglion stimulating action, such as acetylcholine, nicotine and lobeline. The carotid body-stimulating action of each of these substances is suppressed or abolished by hexamethonium, but the abolition of this ganglion-like sensitivity does not interfere with the response to anoxia or cyanide. The site of action of acetylcholine, nicotine and lobeline remains obscure, but it is apparently not involved in transmission of glomus cell responses.

REFERENCE

Euler, U. S. v., Liljestrand, G. & Zotterman, Y. (1939). *Skand. Arch. Physiol.* **83**, 132.

The mode of action of tetraethylpyrophosphate at the cat's neuromuscular junction. By W. W. Douglas and W. D. M. Paton. *National Institute for Medical Research, Mill Hill, N.W.* 7

Tetraethylpyrophosphate (TEPP) is known to cause neuromuscular block which is generally attributed to its cholinesterase-inhibiting properties but which has also been ascribed to a direct action by the drug at the neuromuscular junction.

Using the technique described by Burns & Paton (1951), we found that intravenous injections of TEPP (500 μg./kg.) were often followed by fluctuating depolarizations of the end-plate regions of the cat's gracilis. When depolarization did not occur spontaneously, it rapidly followed stimulation of the tied motor nerve to gracilis. In general, the degree of depolarization depended on the activity of the motor nerve, excited either by injury or electrically. With bigger doses of TEPP depolarization always occurred, and was large and persistent. The depolarizations observed were fully adequate to account for the neuromuscular block produced by TEPP. They were similar in magnitude to those produced by blocking doses of acetylcholine or decamethonium, but differed in the time of onset and the course of the depolarization. In addition, TEPP differs from these directly acting substances by its failure to elicit a twitch of the tibialis when injected close arterially, even in a dose which was followed by a complete and prolonged neuromuscular block. These results indicate that the depolarizing action of TEPP is indirect.

The only fact suggesting a direct action is that TEPP, in yet larger doses (10 mg./kg.) given intravenously, can still evoke a slowly developing depolariza-

tion of a muscle denervated 4–6 days previously to exclude liberation of acetylcholine at the nerve endings of the muscle. But this depolarization is, in fact, due to the accumulation of acetylcholine from other sites, particularly from the bowel. Blood from animals receiving these large doses of TEPP was found to contain as much as 40 mμg. acetylcholine/ml.

Neuromuscular block caused by TEPP is due, therefore, to acetylcholine produced either locally at motor nerve endings or at sites remote from the muscle. No direct action by TEPP was detected or need be postulated to account for its neuromuscular effects. These experiments emphasize, in addition, the care that must be taken in interpreting the local effects of anticholinesterases when these drugs are given systemically.

REFERENCE

Burns, B. D. & Paton, W. D. M. (1951). *J. Physiol.* **115**, 41.

The influence of insulin hypoglycaemia on pepsin secretion in Heidenhain pouches in dogs. By PAMELA A. BURSTALL and B. SCHOFIELD. *Department of Physiology, The Medical School, King's College, Newcastle upon Tyne*

Heidenhain pouches in fasting dogs are washed out every 15 min. with warm N/200-HCl. The pepsin in the washings is estimated by a modification of the method of Hunt (1948).

Heidenhain pouches in fasting dogs show a high resting secretion of pepsin. Following the administration of 1·5–2 units of insulin/kg. intravenously, there is a variable slight increase in pepsin output. This increase is seen only in the insulin-positive pouches, i.e. those which produce acid secretion in response to insulin hypoglycaemia. It is complete within ½–1 hr. and is followed in all cases by a marked depression of pepsin output. Minimum levels are attained between 1½ and 3½ hr. after insulin, and for a group of fifteen experiments on four dogs the mean output over this period was reduced to one-fifth of the previous resting level ($-80{\cdot}8 \pm 2{\cdot}2\%$). The lowest pepsin levels occur about 30 min. later than the maximum degrees of hypoglycaemia, and there is a tendency for the pepsin output to recover as the blood-sugar level rises.

A series of experiments on three Heidenhain pouch dogs has been carried out to determine whether or not insulin would produce a similar inhibition of the pepsin secretion in response to a stimulant drug. Two doses of 0·25 mg. 'Mechothane' (urethane of β-methylcholine hydrochloride) are given intravenously at intervals of 2½–3½ hr. The total pepsin produced by each dose is estimated and the difference between the two expressed as a percentage of the first. Eight control experiments showed a mean difference of $+0{\cdot}1 \pm 8{\cdot}2\%$.

In six experiments in which 1·5–2 units insulin/kg. was given intravenously between the two doses of 'Mechothane', the mean difference was $-63{\cdot}2 \pm 2{\cdot}4\%$. This is a significant difference compared with the controls ($t = 6{\cdot}5$, $P = < 0{\cdot}01$).

We have previously suggested that the acid response to insulin hypoglycaemia shown by some Heidenhain pouches represents a balance between stimulant and depressant effects (Burstall & Schofield, 1951). The same appears to be true of pepsin secretion, but the inhibitory effect is here predominant.

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REFERENCES

Burstall, Pamela A. & Schofield, B. (1951). *J. Physiol.* **115**, 19 *P*.
Hunt, J. N. (1948). *Biochem. J.* **42**, 104.

The secretion of the denervated adrenal medulla. By MARTHE VOGT

The effect of duodenal contents on abomasal motility in goats.

By A. G. SINGLETON. *Laboratory of Physiology, Department of Physiology and Histology, The University of Liverpool*

In the dog and man there is evidence that a major factor influencing the rate of gastric emptying is the depression of gastric tone and propulsive motility resulting from the presence in the duodenum of acid, hypertonic solutions, or fat and protein digestion products. The mechanisms involved are reflex (the 'enterogastric reflex') and hormonal (enterogastrone). The experiments to be described show that in goats depression or inhibition of abomasal motility may be produced by the duodenal instillation of acid, or fat and protein digestion products, indicating the existence of a mechanism similar to that in man and the dog for control of gastric emptying in the ruminant.

Goats were provided with abomasal and duodenal cannulae and abomasal motility recorded by the introduction into the pyloric region of a balloon attached to a water manometer writing on a smoked drum. All solutions introduced into the duodenum via the cannula were at body temperature and except in the case of fats the volume was 25 ml. The introduction of N/100 HCl (pH 2·1) caused immediate inhibition of abomasal contractions lasting 2–10 min. Proteins and their breakdown products in 2% solution or suspension gave less consistent results but inhibition of varying duration was observed in about 50% of experiments. Isotonic glucose solution was rarely effective but stronger solutions (20–30 %) caused inhibition in some experiments. Fat (olive oil or ground-nut oil emulsified with gum acacia) consistently produced inhibition. 5 ml. of a 50 % emulsion was always effective, sometimes immediately, but more often

after a short latent period. Usually periods of inhibition alternated with bursts of activity for 1–2 hr. before the pattern of contractions returned to normal. Isotonic saline had no effect; 2% saline *increased* abomasal activity. Duodenal drainage (open duodenal cannula) increased abomasal activity. These findings prove that the contents of the duodenum influence abomasal activity in the goat; they do not show to what extent the influence is reflex or hormonal in character.

Spontaneous and reflexly elicited contractions of reticulum and rumen in decerebrate sheep. By AINSLEY IGGO.* *Rowett Research Institute, Bucksburn, Aberdeenshire*

The cycle of contractions of the reticulum and rumen in unanaesthetized sheep is periodic, recurring every 50–70 sec. (Phillipson, 1939). A sharp double contraction of the reticulum is followed immediately by a contraction of the rumen.

Cyclic contractions of the reticulum and rumen have been observed in sheep decerebrated, under pentobarbital anaesthesia, at the level of the superior colliculus, and in one preparation after additional transection of the spinal cord at level of second thoracic vertebra. Records of these movements obtained from lightly inflated balloons, placed within the cavity of the organ, were made by water manometer and more recently by a condenser pressure-head, converter unit and oscillograph. Previous observations showed that contractions of the reticulum could be obtained by stimulation of the central end of the cut cervical vagus in anaesthetized ewes (Duncan & Phillipson, 1950, personal communication) or by stimulation of the central end of the cut abomasal branch of the abdominal vagi in decerebrate calves (Comline & Titchen, 1950, unpublished observations).

Reflex contractions of the reticulum, similar to normal ones, were produced by tetanic stimulation of the central end of a transected thoracic vagal trunk or the central end of vagal branches to the abomasum. Furthermore, stimulation adequate to elicit one response of the reticulum in some instances was sufficient to induce contractions, at intervals not greatly different from normal, which continued for periods of 5–50 min. In four instances reticulum and rumen contractions have appeared spontaneously immediately after decerebration, without stimulation of vagal branches, and persisted for periods up to 45 min.

In preparations in which a regular series of contractions was present the central control of the contractions was demonstrated by complete or partial block of the cervical vagi (*a*) by 2% procaine hydrochloride, (*b*) by cooling and (*c*) by pressure, which resulted in reversibly graded depression or complete

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inhibition of the contractions. Section of the cervical vagi caused an immediate cessation of activity, while in the preparation with the spinal cord transected, with vagi intact, contractions could be elicited.

It is postulated that there is a region in the brain of the sheep caudal to the intercollicular plane which can maintain co-ordinated activity of the reticulum and rumen. This region receives vagal afferent impulses which can influence its activity and reticulum and rumen contractions can be 'triggered' by vagal afferent inflow. The name 'Reticulo-ruminal motor centre' is proposed for this region.

REFERENCE

Phillipson, A. T. (1939). *Quart. J. exp. Physiol.* **29**, 395.

Effect of some choline esters upon the rumen and reticulum of sheep. By DOROTHY L. DUNCAN. *Rowett Research Institute, Bucksburn, Aberdeenshire*

Quin & van der Wath (1938) observed that in sheep, the intravenous injection of acetylcholine caused an immediate and complete inhibition of ruminal movement. Clark (1950) found that both carbaminoylcholine and physostigmine stimulated movements. Neither distinguished between pressure changes due to the reticulum and the rumen, both organs being treated as one.

The present observations were made by recording the reticulum and rumen separately, introducing balloons into each organ and recording pressure changes. The inhibitory effect of intravenously injected acetylcholine on both rumen and reticulum was confirmed. Carbaminoylcholine chloride, 0·01–0·02 mg./kg. subcutaneously, increased tone and frequency of contraction in the rumen. In the reticulum, however, dosage at the upper end of the range inhibited movement, after a brief initial stimulation. Increased reticular activity was represented by greater amplitude of contractions, but not by increased frequency. Dosage at the lower end of the range occasionally produced stimulation only, not succeeded by inhibition.

Subcutaneous injection of physostigmine sulphate, 0·3–0·6 mg./kg., caused, after some 40 min. delay, a similar effect upon the rumen. The reticulum generally exhibited marked increase in amplitude, though not in frequency, of contractions, followed on a few occasions by inhibition. Atropine sulphate, 0·3–0·6 mg./kg. subcutaneously, depressed motility in both reticulum and rumen.

As both carbaminoylcholine and physostigmine have similar actions, it may be postulated that small concentrations of choline ester in the reticulum are stimulatory, but higher concentrations inhibit, as in the heart (Burn & Vane,

1949). With the rumen, only stimulation was observed at the dose levels employed. The inhibitory effect of injected acetylcholine on both organs is surprising, but its somewhat prolonged action suggests that this result may be due to indirect effects.

REFERENCES

Burn, J. H. & Vane, J. R. (1949). *J. Physiol.* **108**, 104.
Clark, R. (1950). *J. S. Afr. vet. med. Ass.* **21**, 49.
Quin, J. I. & van der Wath, J. G. (1938). *Onderstepoort J. vet. Sci.* **11**, 361.

A method for determining the nutritive value of a protein by its effect on liver protein. By KATHLEEN M. HENRY, *National Institute for Research in Dairying, University of Reading*, H. W. KOSTERLITZ, *Department of Physiology, University of Aberdeen* and M. H. QUENOUILLE, *Department of Statistics, University of Aberdeen*

Campbell & Kosterlitz (1948) have shown that the nutritive value of a protein can be assessed by its effect on the amount of cytoplasm or protein in the liver cells of adult rats.

The method has been further developed and adapted for use with young rats. To compare three proteins, six litters of six male rats each, maintained on their stock diet until they weigh about 100 g., are used. The rats are randomized in a Latin square design, the rows and columns of which represent the litters and weight orders of the animals. Two rats of each litter are given the diets containing the test proteins in concentrations of 5–8 and 10–16% respectively for 10 days; the rats are then killed and the total N content of their livers is determined. The diets are based on a protein-free diet of the following percentage composition: sugar 12, potato starch 10, margarine fat 10, salts 4, rice starch 64. A daily supplement of pure vitamins is given. The test proteins are substituted for an equivalent amount of rice starch. The diets are given at the rate of 9 g. (about 36 kg.cal.)/day/100 g. initial body weight. The regressions of mg. total liver N/100 g. initial body weight on mg. N intake/day/100 g. initial body weight are calculated for each protein. The ratio of the slopes of the regression lines of two proteins is taken as the ratio of their nutritive values.

To obtain a more accurate test of the linearity of the regression lines, an additional group of litter-mate rats fed on the protein-free diet for 5 days was included in the design in some experiments.

With ether-extracted whole dried egg as a reference protein, satisfactory agreement was obtained with the growth method (Osborne, Mendel & Ferry, 1919) and with the balance-sheet method (Mitchell, 1924; Mitchell & Carman, 1926) for casein and for the proteins of milk and flour.

REFERENCES

Campbell, R. M. & Kosterlitz, H. W. (1948). *J. Physiol.* **107**, 383.
Mitchell, H. H. (1924). *J. biol. Chem.* **58**, 873.
Mitchell, H. H. & Carman, G. G. (1926). *J. biol. Chem.* **68**, 183.
Osborne, T. B., Mendel, L. B. & Ferry, E. L. (1919). *J. biol. Chem.* **37**, 223.

Further observations on the chronotropic action of L-noradrenaline. By I. R. INNES and H. W. KOSTERLITZ

Action of adrenaline on some mechanical properties of mammalian muscle. By M. GOFFART and J. M. RITCHIE

The value of various electrodiagnostic procedures in human nerve injury. By A. E. RITCHIE

Blood oxygen levels and foetal asphyxia. By J. WALKER

The effect of cortisone and ACTH on the behaviour of adrenal homografts in the rat. By M. F. A. WOODRUFF

Absorption from the intestine of the rat with blind self-filling intestinal pouch. By VIVIEN PIRIE and D. W. TAYLOR

A modified Folin-Malross technique for the estimation of blood-sugar, particularly suitable for low values and micro-work. By F. W. LANDGREBE and K. A. MUNDAY