

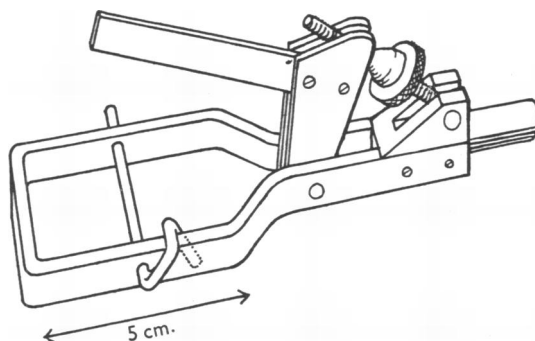
PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY
PHYSIOLOGICAL LABORATORY, CAMBRIDGE

19 *May* 1951

A head-holder of simple construction. By T. D. M. ROBERTS.

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The disadvantages of the conventional Czermak head-holder were discussed by Bishop, Brown & Kearney (1951), but the modified pattern which they described has certain disadvantages of its own. For instance, the moving member of their head-holder is of a complex shape and is subject to inconvenient shearing stresses which make fabrication difficult except in a very well-equipped workshop.



In the new pattern presented, the moving member works on a different principle, the screw applying pressure instead of tension, as will be seen in the diagram. The parts are all very readily constructed, and it is believed that all the advantages claimed for earlier models are retained. In addition, the head-holder may be supported in a standard bosshead by the short piece of $\frac{3}{8}$ in. rod provided.

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Effects of temperature-shock on bull spermatozoa. By J. L. HANCOCK

A summation technique for detecting small signals in a large irregular background. By G. D. DAWSON. *Neurological Research Unit, Medical Research Council, National Hospital, Queen Square, London, W.C. 1*

The cerebral responses to nerve stimulation which can be picked up from the scalp in man are small in relation to spontaneous activity of scalp muscle and brain. They have been detected by superimposing a number of records; this emphasizes regular features, while the irregular background appears as a diffuse thickening of the whole trace (Dawson, 1947, 1950). In a majority of subjects,

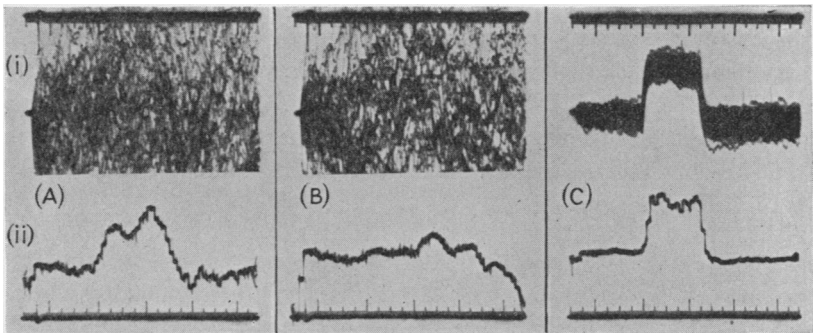


Fig. 1. An experiment to detect cerebral responses when the left ulnar nerve was stimulated at the wrist once per second. The upper line of traces shows sets of 55 records superimposed and the lower line the averages of these given by the machine. In A, from the contralateral scalp, there was one electrode on the midline and one over the right central sulcus. In B, from the ipsilateral scalp, the record was taken from the same midline electrode and one over the left central sulcus. In C is shown the result of making the electrode over the central sulcus positive to that on the midline by $5 \mu\text{V}$. The largest spikes in the time scales show intervals of 20 msec., and the stimulus was applied 5 msec. after the start of each sweep.

however, the level of unwanted activity is too high for superimposition to show clearly the form and distribution of the responses. A much greater degree of discrimination may be obtained if instead of superimposing the records they are added to obtain the mean. Waves not regularly related to the stimuli are insignificant in the mean curve. To see how valuable this method was likely to be sets of records were measured by hand and averaged. When the results were plotted the responses became progressively clearer as the number of records added increased from 5 to 40.

The apparatus to be demonstrated automatically adds successive potential waveforms from the scalp during a series of stimuli and it displays the sum continuously. The additions are made in a bank of condensers to which the signals are distributed by a multi-contact switch rotating synchronously with the recording sweep. In the present apparatus, which is far from ideal, there

are 60 condensers and each sweep lasts 100 msec. Fig. 1 compares records obtained simultaneously by this means and by superimposition.

I would like to thank Dr J. N. Hunt who first suggested that an additive technique would be of value and that a condenser storage system might be used.

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Simultaneous determination of oxygen content and gas-combining capacity of blood or haemoglobin solutions by gasometric technique. By A. B. OTIS* and F. J. W. ROUGHTON. *Department of Colloid Science, University of Cambridge*

Hitherto it has been usual, in Van Slyke gasometric procedure, to determine oxygen content and gas-combining capacity on successive samples of blood (or haemoglobin). The method presently demonstrated enables both of these estimations to be made on the same sample, thus leading to (a) improved accuracy, (b) elimination of errors arising from changes in combining capacity, which have been shown to occur with time in blood outside the body (Roughton, Darling & Root, 1944; van Slyke, Hiller, Weisiger & Cruz, 1946).

The principle of the method consists in using an excess of carbon monoxide to release the oxygen from combination with haemoglobin (as suggested by Claude Bernard) and measuring in a single experiment both the CO absorbed by the haemoglobin and the oxygen liberated therefrom, by means of standard gasometric apparatus. The principle has been applied in two ways.

(1) With the Haldane gas analysis apparatus (requiring 5–10 c.c. of blood or the equivalent amount of haemoglobin solution). The blood sample is measured into a vacuous tonometer with three-way taps at either end and about 100 c.c. capacity. A volume of CO, V_1 (in the range 9.3–10 c.c.), is exactly measured in the gas burette of the 10 c.c. Haldane gas-analysis apparatus and quantitatively delivered into the tonometer, which is then shaken in a water-bath for 40 min. The gas phase in the tonometer is then brought to atmospheric pressure by connecting it with a mercury reservoir, and transferred back to the Haldane apparatus, where, after absorption of CO_2 , its volume V_2 is measured. The O_2 is next absorbed and the final volume V_3 measured. The O_2 content of the original blood is calculated from $V_2 - V_3$, suitable solubility corrections being inserted. The CO absorbed by the blood measures its gas-combining capacity, provided that the original CO content of the blood is negligible, and is similarly calculated from $V_1 - V_3$.

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(2) With a combination of the van Slyke constant-volume apparatus and the Scholander gas-analysis apparatus (Scholander, 1947). This procedure requires 1–2 c.c. of blood or the equivalent amount of haemoglobin solution. The blood is measured into the chamber of the van Slyke apparatus and is followed by about 1.5 c.c. of CO (free of O₂). The mercury is lowered to the 50 c.c. mark and the apparatus shaken for 10 min. with an opaque cover over the chamber. The pressure of the gas phase is then measured at the 2 c.c. mark (p_{12}), and an aliquot part of the gas is transferred to the Scholander gas-analysis apparatus, wherein its oxygen percentage is measured to ± 0.02 vol. %. The remainder of the gas is quantitatively expelled from the chamber of the van Slyke apparatus, and a second pressure reading is taken (p_{22}). The oxygen content of the blood is readily calculated from $p_{12} - p_{22}$, and the percentage oxygen as measured by the Scholander apparatus. The CO content of the CO-saturated blood remaining in the van Slyke chamber is finally determined by the method of Roughton & Root (1945).

This second method, which we have developed more recently, is proving not only more economical of blood, but also appears to be much more expeditious.

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The effect of vitamin A on skeletal tissue cultivated *in vitro*. By HONOR B. FELL, *Strangeways Research Laboratory, Cambridge*, and E. MELLANBY, *Nutrition Building, National Institute for Medical Research, Mill Hill, London*

The object of this study was to see whether hyper- and hypovitaminosis A acted directly on skeletal tissue grown under the simplified conditions of tissue culture, and if so, what the effects were. The following points will be demonstrated:

Hypervitaminosis A

(1) In one series of experiments, the rudiments of the limb-bones were removed from 6-day chick embryos; at this stage the limb-skeleton consists of very early, unossified cartilage. The cartilages were cultivated by the watch-glass method; one of each pair was placed in normal medium (3 parts plasma : 1 part embryo extract) and the other in medium containing about 1400 i.u./100 ml. of vitamin A. For the first 2–3 days the two sets of explants grew equally well, the diaphysial cartilage cells hypertrophied and periosteal bone was formed, but the experimental rudiments were more slender than

their controls. After 4-7 days' cultivation, the bones subjected to hypervitaminosis A became rather flabby, a constriction appeared at the junctions of the shaft with the epiphyses which finally were completely cut off from the diaphysis, and the rudiments began to shrink; the soft tissue surrounding the cartilage continued to grow actively. The controls remained normal and often trebled their length in a week.

(2) The limb-bones of late foetal mice were treated in the same way. These rudiments were well developed when explanted, with a stout shaft of bone containing marrow, and large cartilaginous ends, but they were very susceptible to the action of hypervitaminosis A. After 4 days' cultivation in a medium containing about 2000 i.u./100 ml. of vitamin A, the cartilaginous ends began to shrink rapidly, the bone became greatly rarified but the surrounding soft tissue grew profusely. By the 7th day the bone was reduced to a fraction of its original length and after 10 days it had sometimes vanished completely, leaving only a few crumbs of debris scattered in a sheet of actively growing and migrating cells.

These changes were not associated with cell degeneration, and the more healthy and active the tissue appeared, the more severe were the effects of the hypervitaminosis. Although the action of excessive vitamin A on these bones is destructive, it depends on the viability of the tissues. Thus heating the bones at 45° C. for 15 min. destroys their power to grow and also their susceptibility to excess vitamin A. Heating at 40° C. for 15 min. leaves both actions intact.

The effect on foetal mouse bones of plasma from a fowl suffering from hypervitaminosis A was compared with that of normal plasma to which pure vitamin A acetate had been added, so that the amount of vitamin A in the two plasmas was approximately the same. The results showed that the 'natural' hypervitaminosis acted on the explants much more slowly than the same degree of 'artificial' hypervitaminosis induced by the pure vitamin A acetate.

It is possible that the different effects of 'natural' and 'artificial' hypervitaminosis A plasmas are due to the different physical conditions of the vitamin A in the plasma. In the 'artificial' plasma much of the added vitamin A can be directly extracted by shaking the plasma with petrol ether. This does not happen in the 'natural' high A plasma, which requires shaking first in 50% alcohol before it is extractable with petrol ether. Most of the vitamin A in the 'artificial' plasma is in the fat-soluble form, whereas under natural conditions the vitamin A is probably in a water-soluble form, and may be in combination with a protein, and this combination has first to be broken down by alcohol before the vitamin A becomes soluble in petrol ether (Dzialoszynski, Mystkowski & Stewart, 1945).

Hypovitaminosis A

The leg-bone rudiments of 6-day chick embryos were cultivated in plasma from vitamin A-deficient fowls. The rudiments from some embryos showed a pronounced effect; chondroblastic hypertrophy and ossification were retarded and the shafts were much shorter than those of corresponding controls in normal medium. These results agree with Wolbach's observations (Wolbach & Bessey, 1941) on the effect of A-deficiency on the limb-skeleton of very young animals. The bone rudiments of other embryos, however, were unaffected by the hypovitaminosis. It is thought that this variation in the response of bones from different chicks may be correlated with the amount of vitamin A stored in the tissues at the beginning of the experiment, and this again with the amount of vitamin A and carotene in the yolk of the original egg. This point is being investigated. It appears as if the younger the animal of one species the greater are the effects of excessive and deficient vitamin-A plasmas on the bones. The effect of hypovitaminosis A on periosteal bone which is so prominent in older growing animals (Mellanby, 1938) has not yet been demonstrated in tissue-culture experiments. The evidence indicates that the vitamin A is a main controlling factor in the development and growth of bone, and that the concentration in the tissues necessary for the proper development in any species of animal must be within fairly narrow limits.

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Automatic recording skin thermometer. By P. A. G. MONRO

Transport of radioactive potassium by current through a nerve membrane. By A. L. HODGKIN and A. F. HUXLEY

Distribution of radioactive ions in sheep's blood. By T. I. SHAW

Spatial aspects of excitability for a single fibre in an undissected nerve trunk. By J. J. LUSSIER and W. A. H. RUSHTON

Ejection of milk induced by electrical stimulation of the supra-opticohypophysial tract in the rabbit. By B. A. CROSS and G. W. HARRIS

Determination of ionic fluxes in frog's toe muscle. By R. D. KEYNES

The excretion of the D- and L- isomers of amino-acids in the urine.

By R. F. CRAMPTON, Q. H. GIBSON and D. H. SMYTH. *Department of Physiology, University of Sheffield*

From the work of Pitts and others it is known that active reabsorption of amino-acids occurs in the renal tubules of the dog (see Pitts, 1943). Since Gibson & Wiseman (1951) found that the L-isomer of a number of amino-acids is absorbed by the intestine preferentially to the D-isomer, it was of interest to determine whether preferential absorption of the L-isomer also takes place in the renal tubules.

Cats, anaesthetized with pentobarbitone sodium, were eviscerated and the bladder cannulated through the urethra. An intravenous infusion of a 4% solution of a racemic mixture of amino-acid at pH 7.0 was given, the total amount being approximately 1 g./kg. body wt. Urine and blood samples were subsequently collected and analysed for the D- and L-isomers, using the methods described by Gibson & Wiseman.

When DL-alanine was injected it was found that more of the D-isomer than of the L-isomer appeared in the urine, the ratio of the amounts of D-isomer to L-isomer (D/L ratio) in three experiments being 1.8, 3.4 and 2.2 respectively. This suggested a preferential absorption of the L-isomer by the renal tubules. A possible explanation, however, was that the D-isomer was present in higher concentration in the blood due to preferential metabolism of the L-form by the tissues. That this was not the case is shown by the fact that the concentration of L-isomer in the blood was actually higher than that of the D-isomer, presumably due to the greater urinary excretion of the D-isomer. In the experiment where the D/L ratio in the urine was 3.4, the average D/L ratio in the plasma during the collection of the urine sample was 0.62. Similar results were obtained after injection of DL-histidine.

The results indicate that for the two amino-acids tested, alanine and histidine, the rate of reabsorption of the L-isomer in the renal tubules is greater than that of the D-isomer and show that a stereochemically specific process is associated with reabsorption of amino-acids.

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The nature of intestinal maltase. By ENID E. BACON, J. S. D. BACON, E. W. CLARKE and D. H. SMYTH. *Departments of Biochemistry and Physiology, University of Sheffield*

Blanchard & Albon (1950) and Bacon & Edelman (1950) have shown by the use of paper partition chromatography that yeast invertase preparations produce from sucrose not only glucose and fructose but substances with smaller

R_F values than sucrose, and thus presumably of higher molecular weight than sucrose. They suggested that one of these substances might be a trisaccharide, which could arise by the transference of a fructose residue from one sucrose molecule to another. It was of interest to see whether the intestinal enzymes splitting disaccharides have analogous actions. Fluid was obtained from Thiry-Vella loops in dogs, and incubated with maltose. Enzymic action was stopped by raising the temperature quickly to boiling-point and the solution chromatographed without further treatment, using butanol-acetic acid (Partridge, 1948) as developing solvent, and benzidine-trichloroacetic acid (Bacon & Edelman, 1951) as spraying reagent. From a 5% maltose solution, the intestinal fluid was found to produce not only glucose, as would be expected, but also a number of reducing substances with R_F values smaller than that of maltose and comparable with those of the short-chain dextrans. Extracts made from the mucosa of the small intestine, by grinding it with sand and about three times its weight of water and removing the solids by centrifugation, showed much greater activity in both respects. On prolonged incubation of maltose, either with fistula fluid or with mucosal extract, the final product was mainly glucose, and did not include maltose, nor the additional substances formed in the early stages of the reaction. The results suggest that the enzymic splitting of maltose in the intestine is not a simple hydrolysis, and that 'maltase activity' may involve not only production of glucose but in addition formation of compounds of higher molecular weight than maltose, possibly by transference of glucose residues from one maltose molecule to another (cf. the 'amylomaltase' of *Escherichia coli*; Monod & Torriani, 1948).

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A rat heart-oxygenator preparation for use in isotope experiments.

By E. W. CLARKE. *Department of Physiology, University of Sheffield*

An isolated heart preparation most suitable for routine isotope experiments should be small, to permit economical use of isotope materials, and compact, to simplify precautions against laboratory contamination. The following apparatus has been designed for use with the rat or guinea-pig heart. The lungs are replaced by a gas 'lift' oxygenator using glass-wool as a defrothing device. The aorta is ligated and the whole cardiac output diverted to the coronary vessels, thus avoiding the usual artificial systemic circulation. This enables the

apparatus to be reduced to a single unit consisting of a reservoir and heart chamber connected by the lift tube. The circulating blood volume is approximately 5 ml. and the survival period from 2 to 3 hr.

The animal is anaesthetized, respiration maintained by means of a small ventilation pump, and heparin injected. The reservoir of the apparatus bears a short cannula which is introduced into the left atrium. The reservoir fills with pulmonary venous blood as the ligature is gradually tightened round the aorta. The pulmonary vessels are occluded by another ligature and the heart cut free from the mediastinum. The reservoir, with heart attached, is transferred to the heart chamber. The blood then circulates from the reservoir to the left atrium, left ventricle, aorta, coronary vessels, right atrium, and after leaving the heart through the cut venae cavae is carried by the gas-lift back to the reservoir. The gas stream from the oxygenator is led through a carbon dioxide absorber and recirculated in a closed system by a small pump. The volume change in the system (i.e. oxygen consumption) is measured, the whole apparatus being immersed in a constant-temperature water-bath at 37° C.

The average rate of oxygen consumption for the rat heart under these conditions is 7 ml./g./hr. Acetate added to the preparation disappears at rates up to 12 mg./g./hr. Using ¹⁴C-labelled acetate, it can be shown that as much as 50–70% of the respiratory carbon dioxide may be derived from added acetate. This corresponds to a rate of acetate oxidation of approximately 5 mg./g./hr., which is considerably higher than the values obtained in similar experiments using the cat heart by Lorber, Lifson, Wood & Barcroft (1946).

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The effect of increased blood acidity on respiration and on the response to CO₂. By J. H. WOLSTENCROFT

Changes in the blood flow in the human skin and muscle following sympathectomy. By R. S. DUFF. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London, S.E. 1*

Sympathectomy has been shown, in the human subject, to cause vasodilatation in the hand and foot (representing mainly skin circulation), which reaches a peak some 24–48 hr. after operation (Barcroft & Walker, 1949; Lynn & Barcroft, 1950). Thereafter the blood flow subsides gradually, till towards the end of the first week it has returned to near the resting level.

Simultaneous measurement of the blood flow in the forearm (mainly muscle) and in the hand (skin) of sympathectomized limbs now shows (1) that the peak dilatation in the forearm occurs within 12 hr. of operation, at a time somewhat before the peak dilatation in the hand; (2) that the increase in blood flow in the forearm is less than that in the hand; and (3) that the forearm vasodilatation is of shorter duration than that in the hand.

The delay in the appearance of the peak dilatation in the hand may be attributable to the earlier shunting of the increased blood flow through the vascular bed of the forearm. The smaller peak dilatation in the forearm may be related to the fact that the blood vessels in skeletal muscle are normally under less complete nervous control than the skin blood vessels. Despite these differences, the recovery of tone follows a similar pattern in the vascular beds of skin and muscle. This suggests that similar factors may be responsible for the regain of tone in both groups of vessels.

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The part played by the nervous system in the response to cold of the circulation through the finger tip. By A. D. M. GREENFIELD, J. T. SHEPHERD and R. F. WHELAN. *Department of Physiology, The Queen's University of Belfast*

Lewis (1930) failed to find the normal vasodilatation on immersing a finger tip in water at about 0° C. in persons in whom the sensory nerves had been cut and had had time to degenerate. Grant, Bland & Camp (1933), however, found that the response in the rabbit's ear was largely independent of nerves. We have reinvestigated the response of the human finger to immersion in water between 0 and 6° C. using a calorimetric method (Greenfield & Shepherd, 1950) to obtain quantitative results.

Six cases of peripheral nerve injury have been examined, all of whom had complete anaesthesia of at least one finger, and in three of whom a peripheral nerve had been seen to be completely divided. We confirm Lewis's observations that there is a good vasodilator response in the early days after nerve section.

Between the 30th and 201st days after nerve injury, a sufficient interval for the degeneration of the somatic nerve fibres, the response was 20–50% of the normal size after preliminary immersion in water at 29° C. for 20 min. before insertion into the cold calorimeter. If, however, the preliminary immersion of the anaesthetic finger was at 42° C., a vasodilator response of 50–90% of the normal size was obtained in every case and on every occasion. We have

evidence to show that this was a genuine vasodilatation, and not merely a continuation of the high blood flow induced by the preliminary heating. After preliminary immersion at 15° C. the response was almost absent. The temperature during preliminary immersion made little difference to the response of the normal finger.

We conclude that it is unnecessary to postulate a local axon reflex as the basic mechanism of cold vasodilatation, but that the response is considerably improved if the somatic nerves are intact.

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The influence of sodium γ -resorcylate on corneal wound healing in the guinea-pig. By F. W. CAMPBELL and K. C. WYBAR. *The Institute of Physiology and The Tennent Institute of Ophthalmology, The University of Glasgow*

Sodium γ -resorcylate has been found by Reid, Watson, Cochran & Sproull (1951) to be about ten times as powerful as sodium salicylate in the treatment of acute rheumatism. The clinical effects of this drug resemble those of ACTH and of cortisone. This similarity has been supported by investigations on experimental animals by Buttle (1951) and Prunty (1951).

Standard heat injuries (Campbell, Ferguson & Garry, 1950) were inflicted on the anaesthetized corneae of two groups of guinea-pigs. There were ten animals in each group. One group received daily 25 mg. sodium γ -resorcylate in 0.5 ml. distilled water intraperitoneally, and the control group received only 0.5 ml. distilled water daily intraperitoneally. The degree of epithelial healing was estimated every 12 hr. using a fluorescein solution (Campbell & Boyd, 1950), and the lesions were examined histologically at the conclusion of the experiment.

Sodium γ -resorcylate did not influence the rate of healing during the first 108 hr. By this time fluorescence had ceased in most of the injuries of both groups, indicating restoration of epithelium over the site of injury. Thereafter a significant number of lesions in the group receiving sodium γ -resorcylate began to fluoresce once more. This indicated a failure of maintenance of the epithelium at the site of injury. Histological examination showed a diminished cellular reaction and a thinning of the substantia propria at the injured area in this group as compared with the control group.

There is little doubt that sodium γ -resorcyate impairs the new formation of collagenous tissue in the cornea after injury. Since cortisone is thought also to retard connective tissue formation, here is another indication of similarity in action between these compounds.

We are indebted to Dr J. Reid, of the M.R.C. Chemotherapeutic Research Unit in Glasgow, for a supply of sodium γ -resorcyate.

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Paper-chromatographic study of the secretion of the adrenal cortex in various mammalian species. By I. E. BUSH. *National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W. 7*

In 1943 Vogt described the surprisingly high cortical activity of adrenal venous plasma, and other workers have since made bioassays of this material (Vogt, 1943; Brownell, 1950; Spencer, 1950; Hartmann & Liu, 1950). In order to study the chemical nature of this secretion a paper-partition chromatographic method, which improves on the method previously described (Bush, 1950, 1951), has been developed for steroids and used to analyse extracts of adrenal venous blood drawn from anaesthetized, heparinized animals.

The chromatographic systems used were: benzene/50% methanol; benzene+light petroleum (b.p. 90–110° C.) 1:1 by vol./80% methanol; and benzene+light petroleum 1:2 by vol./80% methanol; equilibrated and run at 32–40° C. The steroids were detected by the method of Markham & Smith (1949).

Large amounts of 17-hydroxycorticosterone (I) and some corticosterone (II) have been demonstrated in dog and cow adrenal effluent (Nelson, Reich & Samuels, 1950; Nelson, Reich & Zaffaroni, 1951; Hechter, 1950), but preliminary results obtained with the above methods show considerable species differences in cortical secretion. The dog and cat secrete mainly I and some II; the ferret roughly equal amounts of I and II; the rat and rabbit mainly, if not entirely, II; the guinea-pig mainly I but also II, 11-dehydrocorticosterone and 11-dehydro-17-hydroxycorticosterone. Small amounts of other compounds present in gland extracts have appeared in the adrenal effluent of dog, rat and rabbit but need further study.

Rough estimations of quantity have been made from these chromatograms. All these species secrete at the high rate indicated by Vogt's experiments, but

in terms of body weight the rat has an exceptionally high rate of secretion. Intravenous injection of ACTH (Armour) increased the secretion rate within a few minutes, provided that the stress of the operation had not already caused a maximal rate.

Work done while holding a Medical Research Council grant.

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The accommodation reflex and its stimulus. By E. F. FINCHAM.

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If a negative lens of, say, 1–2 dioptries is placed before the normal eye of a young subject, distant objects are still seen clearly, the eyes having accommodated to neutralize the effect of the lens. When the lens is removed the accommodation is relaxed. A young hypermetropic subject exerts accommodation to correct his error until a positive lens is placed before the eye, when the accommodation is relaxed. These are reflex actions because the subject is not conscious of a difference in the appearance of the scene which could stimulate a voluntary adjustment. A study of this reaction in fifty-five subjects by objective methods has shown that the change in accommodation is always in the appropriate direction, and is not effected by a trial-and-error method; therefore the brain must be informed whether the light, reaching the retina, is convergent or divergent. It has been found that many subjects do not show the reaction with monochromatic light, and it does not occur unless the eye is allowed to make small scanning movements.

It can be shown that, when the eye turns through a small angle from the point of fixation, the rays of light at the opposite sides of the blur-circles on the retina acquire different degrees of obliquity to the surface. This difference is opposite in direction for myopia and hypermetropia. Thus, through the operation of the Stiles-Crawford effect during scanning, the visual mechanism interprets a difference of light-vergence in terms of a difference of brightness. It is concluded that chromatic aberration and the Stiles-Crawford effect both operate in stimulating the accommodation reflex.

Swimming in very cold water. By E. M. GLASER and G. R. HERVEY.

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It has been claimed that a man loses no more heat in water near freezing-point than he should be able to produce while swimming, and it was suggested that swimming might prolong survival in icy water (Glaser, 1950). In order to find out whether people could indeed swim for some time in very cold water, three men wearing ordinary clothes jumped into a pool at a water temperature of about 2° C. One was a bad swimmer, one a moderate swimmer who was not fit, and the third a good swimmer. All three subjects were gasping for breath within 30 sec. of entering the water, and the first two named had to leave the pool after less than 2 min. because they were too breathless to swim. The good swimmer became gradually less breathless and he swam without difficulty for 9 min. until he was asked to leave the water. It can be calculated that he should have lost some 100 Cal. while he was in the water, and that this should have lowered his average body temperature by about 1.5° C., but measurements of his skin temperature and rectal temperature suggest that such cooling could not have taken place.

The entire skin of all three subjects was very painful while they were in the water, and it became bright red when they left it.

Respiratory distress following immersion in icy water has been described before (Alexander, 1945), and it may be a result of pulmonary congestion (Glaser, 1949).

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A new visual pigment absorbing maximally at 467 m μ . By H. J. A.

DARTNALL. Group for Research in the Physiology of Vision, Institute of Ophthalmology, London

Aqueous digitonin extracts of retinae of the dark-adapted tench contain a photosensitive pigment ($\lambda_{\text{max.}} = 467 \pm 3 \text{ m}\mu.$) in addition to visual violet ($\lambda_{\text{max.}} = 533 \pm 2 \text{ m}\mu.$), the normal scotopic pigment of fresh-water fish (Darnall, 1950). By bleaching the retinal extracts with monochromatic light of long wave-length (600 m μ . or more), the visual violet component may be preferentially bleached without affecting the new pigment; this may then be bleached with white light or monochromatic light of short wave-length (530 m μ . or less).

That the new pigment is not a stage in the bleaching of visual violet is shown by control experiments with an extract of dark-adapted pike retinae.

This contained visual violet only, with precisely the same properties as in the tench extracts.

The new pigment is believed to be a visual pigment because of its similarity in properties to the known visual pigments. Thus it has an absorption spectrum which is independent of pH, gives rise to yellow products on bleaching and has a thermal stability comparable with that of the other visual pigments. Further, its absorption spectrum, when plotted on a frequency scale, is identical in shape with those of the visual pigments, visual purple, visual violet and iodopsin.

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The recovery of manual ability after lesions in the hand area of the sensory cortex in monkeys. By J. COLE and P. GLEES. *Laboratory of Physiology, University of Oxford*

Extending our previous work on the motor cortex (Glees & Cole, 1950) into the sensory areas 3, 1, 2, we have found in monkeys trained to skilled acts that small lesions in the hand area of the sensory cortex cause temporary disuse of the contralateral hand for: (a) skills requiring motor power, (b) discrimination of three-dimensional solids, (c) acts involving dexterity of the fingers and thumb.

The results of such lesions are more severe than those produced by similar injuries in the motor areas; and for a time even the use of the hand for feeding and cage climbing may be abandoned.

Hypotonia as recorded by Peele (1944) was observed, as was also the maintenance of the affected limb in a bizarre posture in which it had been placed.

We are able to confirm Kessler & Kennard (1940), who found that accuracy in the performance of fine motor acts was increased by visual attention.

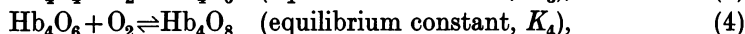
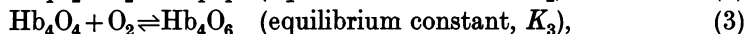
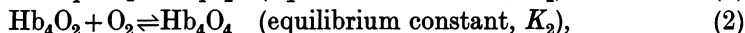
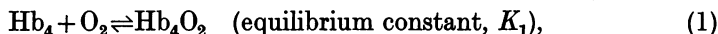
We observed that a very considerable degree of recovery takes place as reported by Ruch, Fulton & German (1938) in their chimpanzees with more extensive lesions; motor power returns almost to normal, the ability to discriminate three-dimensional solids reappears, but a certain slowness of movement and loss of dexterity remain. Efferent connexions of the post-central gyrus can be traced in area 4 anteriorly and into area 5 posteriorly, subcortical projections reach the postero-lateral nucleus of the thalamus. The callosal connexion is well marked and consists of fine fibres.

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The determination of the equilibrium constants of the four intermediate reactions of oxygen with haemoglobin. By R. L. J. LYSTER, A. B. OTIS* and F. J. W. ROUGHTON. *Department of Colloid Science, University of Cambridge*

According to the intermediate compound hypothesis O₂ (and/or CO) reacts with haemoglobin in four successive stages:



Let y = percentage saturation/100, p = oxygen pressure. Application of the law of mass action to equations (1)–(4) leads then to the relation

$$4y + K_1 p (4y - 1) + K_1 K_2 p^2 (4y - 2) + K_1 K_2 K_3 p^3 (4y - 3) + K_1 K_2 K_3 K_4 p^4 (4y - 4) = 0, \quad (5)$$

whence it can be shown that

$$Y = K_2 X + K_2 K_3, \quad (6)$$

where $Y = -[4y + K_1 p (4y - 1)] / \{p^3 K_1 [(4y - 3) + K_4 p (4y - 4)]\}$, (7)

$$X = (4y - 2) / \{p [(4y - 3) + K_4 p (4y - 4)]\}. \quad (8)$$

K_1 is determined separately by special measurements at the extreme bottom of the dissociation curve (Paul & Roughton, 1949, 1951), and K_4 is likewise determined separately by special measurements at the top of the curve (Roughton, 1951). Knowing K_1 and K_4 independently, the values of Y and X can then be calculated from accurate data over the main range of the dissociation curve (10–90% saturation). A plot of Y versus X should give a straight line of slope K_2 , intercepting the Y -axis at a distance $K_2 K_3$ from the origin. K_2 and $K_2 K_3$ are thus separately obtained, $K_2 K_3$ with greater accuracy than K_2 .

This procedure has been applied to solutions of sheep haemoglobin (3–4 g./100 ml.) in $m/5$ -borate buffer, pH 9.1, 19° C., new technique (Otis & Roughton, 1951) having been devised to give higher accuracy than heretofore over the main part of the dissociation curve.

Our thanks are due to Mr H. E. Daniels for help in the statistical analysis of the results.

* Fulbright Research Scholar.

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A comparison between adults, full-term and premature infants in their respiratory response to oxygen. By K. W. CROSS and T. E. OPPÉ. *Physiology Department, and the Paediatric Unit, St Mary's Hospital Medical School, London*

The respiratory behaviour of a group of healthy but premature infants has been studied by the use of the body plethysmograph (Cross, 1949), and gas mixtures (100% oxygen or 15% oxygen in 85% nitrogen) have been supplied according to the technique of Cross & Warner (1951).

The results on full-term infants already reported, and the results obtained by Dripps & Comroe (1947) on the adult human subject, show that the immediate response to 100% oxygen is in all cases a diminution in the minute volume, which is most marked in the group of ten premature infants of 1.8 kg. or less. Subsequent to this initial drop there is a stimulation of respiration in all groups.

With 15% oxygen in the inspired air, both full-term and premature infants respond with an immediate increase in pulmonary ventilation, which is not maintained beyond the second minute in either group. On the other hand, an adult subject has a significant rise of ventilation at the 6th to the 8th minute. When premature infants are given 100% oxygen immediately after 5 min. on 15% oxygen, there is a dramatic fall in the minute volume which is significantly more marked in the group of premature infants of 1.8 kg. and under. This drop can hardly be explained on the basis of CO₂ wash-out during the administration of 15% oxygen (for the over-all hyperventilation is only 2.4% for the 5 min. hypoxia).

It is tentatively suggested that there is an active carotid body reflex in the full-term and premature infant which is apparently more active than in the adult human subject. It also appears that this reflex accommodates or fatigues rapidly in newborn infants, but this accommodation is less complete in the full-term than in the premature baby.

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Relation between glomerular filtration rate and sodium excretion in man. By T. M. CHALMERS, A. A. G. LEWIS and G. L. S. PAWAN. *Department of Medicine, Middlesex Hospital Medical School, London*

Twenty observations were made on eight healthy male subjects during diuresis induced by drinking a litre of 0.15% sodium chloride solution and maintained by drinking as much water as was passed in each 15–20 min. urine collection

period. Once diuresis was established, preliminary observations showed that urine flow, sodium excretion, glomerular filtration rate (g.f.r.) (inulin) and renal plasma flow (PAH) would remain fairly constant over a 1½–2 hr. period for subjects resting in the semi-recumbent posture. When congesting cuffs were applied to the thighs for 30–40 min., g.f.r. fell to an average of 62%, and renal plasma flow to 70% of initial levels. On releasing the cuffs renal plasma flow promptly returned to normal and g.f.r. rose sharply, but usually did not exceed 90% of initial values during the next 45 min. During the period of application of the cuffs both urine flow and urinary sodium concentration fell slightly, leading to a moderate reduction in absolute sodium excretion, to about 65% of initial values. This is in contrast to the striking fall in sodium output produced by a 30–40% reduction of g.f.r. in dogs (Selkurt, Hall & Spencer, 1949; Pitts & Duggan, 1950). Release of the cuffs was not immediately followed by recovery of sodium excretion in spite of the sharp rise of g.f.r.

Little change in heart rate or systemic blood pressure occurred during the application of cuffs, except in one subject who fainted after 30 min. The faint was associated with a large release of antidiuretic hormone (Noble, Plunkett & Taylor, 1950) and was followed by prolonged reduction of g.f.r. and renal plasma flow. An equally long inhibition of diuresis and a similar fall in g.f.r. were produced in another subject by the intravenous injection of 2 units of 'Pitressin'.

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Intrinsic diurnal rhythm in urinary electrolyte output. By J. N. MILLS and S. W. STANBURY.* *Departments of Physiology and Medicine, University of Manchester*

Stanbury & Thomson (1951) have shown that the urinary output of sodium, potassium, chloride and bicarbonate show a marked diurnal rhythm, being low in the night and rising abruptly in the morning. Mills (1951) showed that, under controlled conditions of food and fluid intake, the urinary flow followed a 24 hr. cycle even if subjects lived on a 12 hr. cycle, and that midnight flows remained below midday flows even when subjects had for a week interchanged their habitual a.m. and p.m. activities.

The routine of Mills (1951), i.e. 48 hr. on a precisely repeated 12 hr. cycle of activities, has been followed by five subjects, and the urinary volume, pH, and output of sodium, potassium, chloride and phosphate determined.

Phosphate excretion always rose on waking, but the 8.30 p.m. exceeded the 8.30 a.m. peak.

Excretion of other electrolytes showed a persistence of 24-hourly rhythm, although one subject showed superimposed peaks of potassium excretion on waking.

Urine flow was closely correlated with excretion of sodium + potassium ($r=0.84-0.96$), and high flows were associated with increased concentration. The flow appears to be osmotically determined.

Urinary pH followed a 24 hr. cycle, with midnight to 8 a.m. samples more acid than the corresponding p.m. values, and in addition samples collected during sleep were more alkaline than preceding and subsequent waking samples.

* Beit Memorial Research Fellow.

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The electro-magnetic measurement of blood flow in unopened vessels. By T. G. RICHARDS and T. D. WILLIAMS

Observations on PAH and creatinine clearances in the dog. By R. V. SELLWOOD and E. B. VERNEY

The carotid circulation in the dog and the vascular supply to the hypothalamus. By P. A. JEWELL

Investigations on the localization of the osmoreceptors. By P. A. JEWELL and E. B. VERNEY

Possible factors involved in the control of the glomerular circulation. By R. V. SELLWOOD

An inhibitory component in the gastric response to insulin hypoglycaemia in Heidenhain pouches in dogs. By PAMELA A. BURSTALL and B. SCHOFIELD. *Department of Physiology, The Medical School, King's College, Newcastle upon Tyne*

A high proportion of Heidenhain pouch dogs are insulin-positive, i.e. they produce a small but definite acid secretion in response to insulin hypoglycaemia (Schofield, 1950). Karvinen & Karvonen (1950, personal communication)

report that a high rate of histamine-induced secretion in Heidenhain pouches is depressed by approximately 50% during insulin hypoglycaemia. It seems possible that the secretion produced in insulin-positive animals represents a balance between a stimulant and a depressant effect, and the following evidence supports this point of view.

(1) In insulin-negative Heidenhain pouches, any resting secretion falls off during insulin hypoglycaemia.

(2) In insulin-positive animals, the peak of the acid response is usually attained within 1 hr. of the insulin being given. After this the secretion declines, though the lowest blood-sugar level is not reached till between 1 and 2 hr. The hypoglycaemia continues after the acid response is over, particularly with large doses of insulin.

(3) Dogs with insulin-positive Heidenhain pouches normally secrete acid in response to psychic stimulation. This response is absent if the stimulation is applied in the later stages of insulin hypoglycaemia.

A series of experiments on three Heidenhain pouch animals, two insulin-positive and one insulin-negative, has been carried out to investigate the depressant effect. Using a similar technique for assessment of secretion to that previously described (Schofield, 1950), two doses of 0.25 mg. 'Mechothane' (urethane of β -methyl-choline hydrochloride) are given intravenously at intervals of $2\frac{1}{2}$ – $3\frac{1}{2}$ hr. The total acid produced by each dose is estimated and the difference between the two expressed as a percentage of the first. Nine control experiments showed a mean difference of $+16.4\% \pm 7.8\%$. In six experiments insulin (1.5–2 units/kg.) was given intravenously after the first 'Mechothane' response, and the second dose of 'Mechothane' was given during the later stages of hypoglycaemia when the insulin secretion was nearly or completely over. This series showed a mean difference of $-46.7\% \pm 8.2\%$, which is a significant reduction compared with the controls ($t=5.4$, $P < 0.01$) and is of the same order as that found by Karvinen & Karvonen.

This work has been aided by a grant from the Medical Research Council.

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Noradrenaline and guinea-pig suprarenal glands. By D. M. SHEPHERD and G. B. WEST. *Pharmacology Laboratory, University of St Andrews, Medical School, Dundee*

Euler, Hamberg & Purkhold (1949) and Schuler & Heinrich (1949) have given evidence to show that extracts of suprarenals of guinea-pigs contain adrenaline and noradrenaline, the latter comprising about 24% of the total amines present. We have been unable to confirm this finding. Traces of noradrenaline

have been found in twelve out of forty-one extracts, but the content has never exceeded 5% of the total amines present. In addition, the average total activity in the glands by biological assay and by paper chromatography (127 μ g. adrenaline/g. fresh tissue) is only about one-third of that previously reported by chemical assay (iodine method).

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Muscle performance of rats treated with growth hormone. By
BRENDA BIGLAND and BARBARA JEHRING. *Department of Physiology,
University College London*

Female hooded Norway rats were arranged in pairs of almost identical weight. They were pair-fed, one member of each pair receiving a daily subcutaneous injection of 0.5 mg. of 'pure' growth hormone for 21 days. The preparation used was the 'fraction B' of Wilhelmi, Fishman & Russell (1948). At the end of this time the treated animals were about 20% heavier than the controls.

The rats were then decerebrated and the right quadriceps muscle of each prepared for myographic recording. Single maximal twitches, summated twitches, tetani and rates of fatigue were recorded optically. At the end of each experiment the muscle was removed from the body and weighed. Slides were made of transverse sections of the muscle using a freezing microtome and the average cross-sectional area of the fibres measured with a planimeter.

An increase of 6-12% was found in the cross-sectional area of the fibres from the treated rats. The tensions produced by these muscles, however, when expressed as g. tension per g. muscle wt., were all significantly lower than those produced by the muscles of the control rats. Indeed, the tetanic tensions obtained from the rats treated with growth hormone, even when uncorrected for muscle weight, were significantly lower ($P=0.01$) than those of the untreated rats.

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Electromyographic study of reflex activity of the abdominal muscles of the cat. By MARY CHENNELLS and W. F. FLOYD. *Department of Physiology, The Middlesex Hospital Medical School, London*

Reflex contraction of abdominal wall muscles of cats under chloralose anaesthesia was studied electromyographically. The nerves stimulated to produce the reflex were the central ends of T.13 to L.3 (skin branches), sciatic and

femoral nerves. Reflex contraction obtained by stimulation of a T.13 to L.3 skin branch, either electrically, or mechanically by skin manipulation, can be inhibited by stimulation of the sciatic nerve.

Influence of the thyroid on body temperature of animals.

By M. MAQSOOD. *School of Agriculture, University of Cambridge*

Since the body temperature gives an indication of the physiological state of an animal, the effects of administration of different doses of thyroxine and thiouracil, for short or long periods, on the internal body temperature of some species of animals have been investigated.

Thyroxine when administered in physiological doses did not significantly affect the rectal temperature of the young male rabbit at different ages, but larger doses resulted in a significant increase in the body temperature. A series of experiments shows that there is a relationship between the rise in the internal body temperature and the dose of thyroxine administered in the rabbit. The increase above the normal body temperature of rabbits fed large doses of thyroxine was relatively higher during the summer than during the winter months. Administration of moderate doses of thyroprotein (thyroactive iodinated casein) did not significantly affect the rectal temperature of the young ram.

Continuous thiouracil treatment resulted in a significant decrease in the body temperature of the rabbit and the ram when compared with their respective controls. Simultaneous administration of thyroxine in amounts about equal to the estimated thyroxine secretion rate to the thiouracil-treated rabbits, maintained the rectal temperature at the same level as that of the control animals.

The body temperature of some species of animals appears to be regulated by the thyroid gland, but further work is needed to elucidate the hormonal aspect of the mechanism of heat regulation.

This work was aided by grants from the Agricultural Research Council.

The hormone control of ovulation in the calf. By W. G. R. MARDEN.

School of Agriculture, University of Cambridge

Subcutaneous horse anterior pituitary injections (F.S.H.) given every 12 hr. for 3-4 days can produce follicular development in the calf as early as the first week after birth. After the first few days of extra-uterine life there occurs no further increase in ovarian response to F.S.H. The calf at 3 weeks responds as well as animals at 20 weeks. Ovulation can occur spontaneously in the calf at less than 3 weeks after birth, provided the follicles have been stimulated

by F.S.H. However, the number of ovulations is small when compared with the number of large follicles developed. Intravenous injection of H.C.G. (human chorionic gonadotrophin) or cattle anterior pituitary extracts high in L.H. will not substantially increase the number of ovulations obtained from calves receiving only one series of F.S.H. injections. Superovulation with the formation of multiple corpora lutea can be obtained in the immature calf by repeating the original F.S.H. treatment after an interval of 15 days and then giving a subcutaneous injection of L.H. at the 21st day. The rate of descent of ova in the uterine tube in the calf is not substantially influenced by the age of the animal, but is greatly accelerated by the presence of an active corpus luteum at the time of the L.H. injection.

This work was aided by grants from the Agricultural Research Council.

Exchangeability of muscle potassium. By R. CREESE.

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Early work with ^{42}K , summarized by Krogh (1946), suggested that only a proportion of the intracellular potassium in skeletal muscle could be exchanged with that in the extracellular fluid. This question has been examined in isolated rat diaphragm muscle.

After 2 hr. preliminary equilibration in Krebs's solution (containing 5 mg. Ca^{++} /100 ml.) the muscles reached a steady state with no further net exchange of potassium. The bath fluid was then changed to one of identical chemical composition but in which some of the potassium was present as ^{42}K . The external fluid was frequently renewed, and after various time intervals the muscles were removed and the radioactivity associated with both the muscle potassium and the potassium in the surrounding fluid was determined. This enabled the fraction of muscle potassium which had exchanged to be calculated. Potassium was determined by the phosphotungstate method of van Slyke & Rieben (1944) with an accuracy of $\pm 1.5\%$.

All the potassium appeared to exchange at the same rate, with a half-time at 38°C . of 47 min. (time constant 69 min.) for the combined permeation and diffusion process (Harris & Burn, 1949) in muscles from rats weighing 120 g. Measurements after 5–9 hr. showed that complete exchange had occurred (99.5% , s.d. of 20 observations ± 2.5).

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Contractions of the reticulum of the young goat. By R. S. COMLINE and D. A. TITCHEN. *Physiological Laboratory, University of Cambridge*

Contraction of the musculature of the stomach on stimulation of the thoracic sympathetic trunk has been reported in the cat and dog (Brown, McSwiney & Wadge, 1930), but not in any of the ruminants in which the sympathetic nervous system and adrenaline are generally held to inhibit movements of the stomach.

In young goats, 1-8 weeks old, continuous localized contractions of the reticulum have been observed in both decerebrate and spinal preparations and in animals anaesthetized with chloralose. The contractions remained after cutting both the vagus and the splanchnic nerves and after destruction of the spinal cord in the thoracic and lumbar regions.

The activity of the reticulum was recorded from a slip of the wall about 4 cm. long and 1-1½ cm. wide which remained attached, with a blood and nerve supply, to the posterior pole of the organ. The free end of the slip was connected to a frontal writing lever.

The spontaneous contractions of the reticulum were abolished by the injection of both atropine and 933F, but in several preparations were only reduced and not abolished by the injection of one of these substances. A contraction occurred after the intravenous injection of L-adrenaline and L-nor-adrenaline (2.5-10 µg./kg.). This effect of adrenaline was abolished by 933F (0.4-1.2 mg./kg.), but not by atropine sulphate (0.2 mg./kg.) injected intravenously.

An intense contraction of the slip, and of the rest of the reticulum, occurred on stimulation of either of the cervical vagus nerves. This was abolished by atropine but was not affected by 933F. In contrast, stimulation of a splanchnic nerve or a thoracic sympathetic trunk produced a smaller contraction which was not affected by atropine but was abolished by 933F.

The injection of adrenaline produced a contraction of the reticulum in decerebrate adult goats. No contractions of the reticulum were found in calves up to 10 days old on stimulation of the splanchnic nerves or on the injection of adrenaline.

This work was aided by a grant from the Agricultural Research Council.

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The inhibition of aconitase by the 'active' factor formed from fluoroacetate in poisoned kidney tissue. By W. D. LOTSPEICH, R. A. PETERS and T. H. WILSON. *Biochemistry Department, University of Oxford*

In connexion with the accumulations of citrate induced by fluoroacetate *in vivo* and *in vitro*, Buffa, Peters & Wakelin (1951) have isolated fluorine-containing tricarboxylic acid fractions (called 'active') from guinea-pig kidney homo-

genates treated with fluoroacetate; other evidence of conversion of fluoroacetate to fluorocitrate has been provided by Elliot & Kalnitsky (1950). Studying the first three enzymes involved in the oxidation of citric acid, Buffa, Lotspeich, Peters & Wakelin (1950) found that this 'active' fraction did not inhibit the water-soluble enzymes, aconitase, isocitric dehydrogenase or oxalosuccinic decarboxylase. Further studies in this laboratory by an improved technique have shown definite inhibition of one of these enzymes, namely, aconitase, by a partially purified preparation of this 'active' fraction. By incubating the enzyme with the inhibitor prior to the addition of substrate large inhibitions were observed.

A water extract of pigeon-breast muscle, stabilized with ferrous sulphate and cysteine (Dickman & Cloutier, 1950), was incubated at 17° C. for 30 min. with 'active' fraction (provided by Peters & Wakelin). Citrate was then added to give a concentration of 1 mg./ml. in the mixture and incubation continued at 17° C. After various time intervals samples were withdrawn, deproteinized with trichloroacetic acid, and analysed for isocitric acid enzymatically (Ochoa, 1948). The reverse reaction was also studied by adding isocitrate and estimating citrate under similar conditions.

Both the forward and the reverse reaction of aconitase are inhibited strongly by the 'active' fraction and to a similar extent.

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Local response of the cat's endometrium to progesterone and other steroids. By J. M. ROBSON and A. A. SHARAF

The transfer of carbon dioxide across the placental barrier in the rabbit and in the sheep. By I. MAUREEN YOUNG

Passive characteristics of electrogenic tissue in *Electrophorus electricus*. By D. ALBE-FESSARD, H. M. FERREIRA and A. FESSARD