The effect of oestrone and of insulin on the metabolism of isolated rat uterus. By LEONORA HOPKINSON and MARGARET KERLY. *Department of Biochemistry, University College, London*

It is well established that the addition of insulin *in vitro* increases the glucose uptake of rat diaphragm, and similar findings have been reported for preparations of rat and mouse intestine. The present report describes the effect of injected oestrone and of added insulin on the metabolism of isolated uterus preparations from ovariectomized rats.

Rats were used for experiments 3–8 weeks after bilateral ovariectomy; those animals to be used for the study of oestrone effects were injected subcutaneously with 20 μg oestrone dissolved in alcohol diluted with an equal volume of saline. The uteri were dissected out under nembutal anasthesia and one horn was incubated for 2 hr at 37° C. in 0·4 ml. saline buffer containing 2·6 mg/ml. glucose, the other horn was treated in a similar manner, except that the incubation medium contained 50 μg/ml. insulin. At the end of the incubation period glucose and lactic acid were estimated in the medium.

Uterus from uninjected rats utilized 1·42 mg/g/hr glucose and produced 3·18 mg/g/hr lactic acid, indicating a high rate of aerobic glycolysis, at least in part from preformed carbohydrate. Addition of insulin increased the glucose utilization to 2·1 mg/g/hr but decreased the lactic acid production to 2·77 mg/g/hr. After injection of oestrone, at 24 hr there was a small (not significant) rise in glucose utilization and a fall in lactic acid production (2·11 mg/g/hr); in both cases the effect of added insulin was no longer observed. At 36 hr after injection glucose uptake had fallen below the value for that observed for uninjected rats (0·77 mg/g/hr) and lactic acid production had fallen still further (1·67 mg/g/hr). There was some indication of a return of the insulin effect on glucose utilization, but the increases in uptake observed with insulin were not statistically significant. Our results for the effect of oestrone injection contrast with those of Walaas, Walaas & Löken (1952), who report increases in both glucose uptake and lactic acid production after injection of oestradiol benzoate, but these workers used uterine muscle only.

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A folic acid derivative functioning during cell division. By W. JACOBSON (Sir Halley Stewart Research Fellow). *The Strangeways Research Laboratory, Cambridge*

When cells are exposed to the action of folic acid antagonists they are arrested in the metaphase stage of division and cannot proceed into anaphase. The chromosomes are formed in the ordinary way at the beginning of mitosis but

fail to split. This was shown on normal and leukaemic bone-marrow cells of the human and mouse, and on the intestinal epithelium of the mouse. It was also observed in a variety of cells grown *in vitro*; chick embryo fibroblasts and osteoblasts with their numerous mitotic divisions provided a very useful test material. The action of folic acid antagonists, like the 4-amino folic acid (aminopterin), is almost instantaneous. The inhibitory action of aminopterin can be completely prevented by the simultaneous application of the Leuconostoc Citrovorum Factor (LCF), a 5-formyl-5, 6, 7, 8-tetrahydro derivative of folic acid. This was shown on cells growing *in vitro*. Folic acid itself is unable to counteract the aminopterin effect when applied directly to the cells.

The following conclusions may be drawn from this:

(1) A folic acid derivative (LCF), but not folic acid, is replaced by the so-called folic acid antagonists.

(2) The function of the LCF is essential for the splitting and separation of the chromosomes.

The cells used were unable to convert folic acid into LCF, as treating them with folic acid 1 hr prior to the application of aminopterin does not protect them from the full inhibitory effect of the antagonist. As aminopterin inhibits dividing cells of a type which cannot convert folic acid to LCF, the primary action of the inhibitor appears to be to displace the LCF within the cells, and not to block the conversion of folic acid to LCF.

The technique of direct application of the test substances to cells made it possible to determine the amount of LCF required to prevent the action of the inhibitor (aminopterin) at a given concentration. As this relationship is not a linear one, it may be assumed that the LCF is not present in a free form within the dividing cells.

It is of interest that in the presence of aminopterin (1) the chromosomes form normally during prophase and (2) the appearance of ribonucleoprotein on the chromosomes towards the end of prophase is not disturbed. However, the transfer of ribonucleoprotein from the chromosomes into the cytoplasm, a process normally concurrent with the anaphase separation of the chromosomes (Jacobson & Webb, 1952*a*, *b*), cannot take place when the LCF is replaced by folic acid antagonists (Jacobson, 1952).

Some types of normal cells and mouse leukaemic cells were found to overcome the initial inhibition by a slow conversion of the antagonist into an inactive compound. Thus if such cells are examined 18–24 hr after treatment, they may give the impression of being 'resistant' to the action of the folic acid antagonist.

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Sodium chloride and the response of smooth muscle. By R. J. S. McDowall and A. A. I. Soliman. *King's College, London*

It is well known that after a large dose of histamine has been applied to the isolated uterus in a bath the muscle becomes insensitive or much less sensitive to doses which previously produced a contraction and remains so for some time. If such an experiment is carried out in Krebs solution, and if, during the period of reduced sensitivity, the sodium in the solution is reduced to half, osmotic pressure being maintained by sucrose, the sensitivity rapidly returns, but is reduced again if the sodium in the solution is increased to normal. Increasing the potassium produces similar effects also (Eastman & Cantoni, 1946). Similar results have been obtained in a uterus rendered anaphylactic to egg albumin. If the preparation is placed in a solution containing low sodium the classical tachyphylaxis, or insensitivity (Dale, 1913), passes off and the preparation responds to a subsequent application of egg albumin. We have, however, not been able to obtain constant results with anaphylactic preparations.

It is also found that the duration of the response to histamine is markedly increased by reducing the sodium chloride in Krebs solution while maintaining the osmotic pressure with sucrose.

It is suggested that in normal Krebs solution smooth muscle like cardiac muscle (McDowall & Zayat, 1953), takes up sodium during contraction, and this is, at least in part, responsible for the insensitivity, and that the reduction of the sodium in the bath makes it easier for the muscle to extrude sodium.

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The effect of insulin on the adrenergic amines of blood. By H. Weil-Malherbe. *Runwell Hospital, Wickford, Essex*

A fluorimetric method for the estimation of adrenaline and noradrenaline in blood (Weil-Malherbe & Bone, 1953) has been applied to study the effect of insulin on their levels in the plasma and red cells of human venous blood. The intravenous injection of insulin (0·1 unit/kg) in fasting subjects is followed by an immediate drop of the level of adrenergic amines in plasma which precedes the fall of the blood sugar. Similarly, in the recovery period, the return of the level of adrenergic amines to the norm precedes the rise of the blood-sugar curve. With larger doses of insulin the effect is prolonged, but not increased in intensity. Interruption of hypoglycaemia by oral or intravenous glucose, or by other means, is followed by a return of the plasma adrenergic amines to

or above the normal level. In comatose subjects the peak of the curve coincides with the return of consciousness.

The changes in the plasma level of adrenergic amines are entirely, or almost entirely, accounted for by changes of the adrenaline concentration. Plasma noradrenaline may remain unchanged, or it may vary like adrenaline, but less extensively on a relative scale.

The concentration changes of adrenaline and noradrenaline in red blood cells do not show any very characteristic pattern after insulin injection. In most cases there is a slow and moderate decrease of concentration of both amines, especially when large doses of insulin were used.

The arterial level of adrenergic amines is decreased to a higher extent than the venous level, following an intravenous insulin injection. The arteriovenous difference therefore shrinks, as the mean adrenaline level falls.

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Evidence for a non-specific mechanism of habituation. By E. M. Glaser and G. C. Whittow. *Department of Physiology, University of Malaya, Singapore*

A previous investigation (Glaser, 1953) provided evidence that experimental procedures, such as filling in questionnaires or taking tablets, can give rise to apparent responses in man. When the experimental procedures were repeated at intervals of 7 days, the responses progressively diminished, and this resembled habituation to drugs or to motion sickness observed in the same investigation or in a previous experiment. The evidence also suggested that responses varied according to the subjects' interest in the experiment, and this was again borne out by previous observations on motion sickness.

Further experiments have been carried out to disprove or confirm the above findings. At first 308 students were tested in four different groups, three of which were tested in Singapore and one of which was tested in Belfast by Prof. A. D. M. Greenfield. Carefully designed questionnaires were given to all these students and in each group 68–94% of the subjects recorded at least one symptom. The most frequent symptoms were tiredness, dryness of the mouth, headache and inability to think clearly. One hundred and forty of these students were then given questionnaires repeatedly at intervals of 2–5 days, and the incidence of symptoms decreased by about 20% until the third test, after which it remained at a steady level even if different dummy tablets were given in turn. The subjects were then given a drug in a form that was indistinguishable from the dummies taken in the previous test, and nearly

all subjects had unpleasant symptoms after taking the drug. This appears to have had an inhibiting effect on whatever habituation may have been acquired, because in the following test (when dummies were given again) more symptoms were recorded than before the drug had been taken. In the second test after taking the drug, however, the incidence of symptoms was again the same as it had been before the drug was issued. The results were statistically significant.

No satisfactory explanation is available for the high incidence of symptoms in initial tests. The experiments are compatible, however, with the hypothesis that there is a non-specific reversible mechanism, possibly of cerebral origin, which superimposes a distinct pattern on all kinds of habituation.

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Measurement of capillary filtration rate in the human forearm.

By A. H. Kitchin. *Sherrington School of Physiology, St Thomas's Hospital Medical School*

The rate of formation of tissue fluid in the human forearm when the venous pressure is raised to a known level may be measured by means of the pressure plethysmograph (Krogh, Landis & Turner, 1932; Landis & Gibbon, 1933). The technique depends on the measurement of the volume of the forearm enclosed within the plethysmograph when subjected to external pressure sufficient to collapse the arteries, veins and capillaries. This 'reduced arm volume' is read before and after a period of venous congestion of the arm by the application of a cuff on the upper arm inflated to a standard pressure (40 mm Hg). The method has been modified somewhat to give better temperature control and immobilization of the hand, and to allow blood-flow measurements to be made by venous occlusion plethysmography during the experiment.

The effect on 'reduced arm volume' measurements of variations in the vascular state of the limb has been studied. Reactive hyperaemia and brief venous congestion do not affect the measurement, while repeated applications of pressure cause a steady fall.

Rhythmic contraction of the forearm muscles always resulted in a marked increase in the non-vascular volume of the forearm, whether or not the venous pressure was simultaneously raised by application of an upper arm cuff at 40 mm Hg.

Local heating of the arm to 42° C approximately doubled the rate of formation of oedema, compared with measurements at 34° C.

The effect of release of vasomotor tone was studied by indirect heating and by deep nerve block. In both cases an increase of about 120% in the forearm blood flow was produced. The rate of oedema formation, however, was found to be slightly diminished in these circumstances.

These findings appear to accord with the reported clearance rates of radio-sodium injected into skeletal muscle (Kety, 1949; McGirr, 1952; Rapaport *et al.* 1952).

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The effects of temperature and of *p*-chloromercuribenzoic acid on the reaction $Hb_4O_8 \rightleftharpoons Hb_4O_6 + O_2$ in solutions of sheep blood.

By Q. H. Gibson and F. J. W. Roughton. *Departments of Physiology, University of Sheffield, and Colloid Science, University of Cambridge*

Gibson & Roughton (1952) have previously shown that the value of k_4, the velocity constant for the dissociation of the first oxygen molecule from the fully saturated haemoglobin molecule, may be obtained from observation of the rate of the reaction

$$HbO_2 + CO \rightarrow HbCO + O_2.$$

This work has now been extended to include the effects of temperature and of pH on k_4, yielding an average value of 27 at 19° and pH 9·1, with a temperature coefficient of 3·0 per 10° temperature rise. Changing the pH to 7·1 did not produce any significant increase in k_4 at 19°, but did increase the temperature coefficient to 3·3 per 10°.

Riggs (1952) has shown that the addition of the sulphydryl-group reagent *p*-chloromercuribenzoate affects markedly the form of the oxygen dissociation curve of human haemoglobin, rendering it less strongly inflected. This reagent, at a concentration of 6×10^{-4} M increases k_4 from 27 to 70 at 19° and pH 9·1. The temperature coefficient is decreased from 3·0 to 2·2 per 10° temperature rise. The effects may be reversed substantially by the addition of 10^{-2} M-glutathione. The initial velocity of deoxygenation of haemoglobin in the presence of hydrosulphite is also increased some two-fold by *p*-chloromercuribenzoate. Our work is being extended to solutions of human blood.

The velocity constant, k_4', of the reaction $Hb_4O_6 + O_2 \rightarrow Hb_4O_8$, can be calculated from k_4 and K_4, the equilibrium constant of the reaction. The temperature coefficient, Q_{10}, of k_4' has a normal value of about 2·0, whereas the Q_{10} of k_1', the velocity constant of the reaction $Hb_4 + O_2 \rightarrow Hb_4O_2$, is only

about 1·05, in confirmation of earlier work. The Q_{10}'s of l'_1 and l'_4, the corresponding CO velocity constants, are both about 2.

We have recently measured more accurately than heretofore the overall rate of combination of CO with haemoglobin. A procedure has been developed, with Dr A. Klug's help, for fitting the observed curves in terms of the four intermediate velocity constants, l'_1, l'_2, l'_3 and l'_4. In three different blood samples, the experimental and calculated reaction curves agreed throughout to within less than experimental error (0·5 % saturation). l'_1, l'_2 and l'_3 are all of similar magnitude, but l'_4 is about 20 times greater. The values of the constants in any one sample are probably correct to about 10 % of themselves, but the variations from sample to sample are considerably greater.

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Substances with vasoconstrictor action in rabbit plasma. By J. A. Holgate. *Department of Physiology, School of Medicine, Leeds*

Using an apparatus for perfusion under controlled conditions previously demonstrated to the Society (Holgate, 1949), the rabbit ear has been used as an assay preparation for vasoconstrictor substances appearing in plasma obtained from rabbit blood. Both heparin-plasma and citrate-plasma have been investigated.

(1) Plasma obtained from heparinized rabbit blood (10 i.u./ml.) centrifuged at 900 *g* for 2 hr in glass tubes when assayed against histamine acid phosphate on the ear gave values ranging from 3·0 to 20 μg histamine base/ml. plasma ($n=8$). These figures are high when compared with those quoted by Code (1952). After the addition of mepyramine maleate to the perfusion fluid the action of equivalent doses of histamine acid phosphate was completely abolished, but the activity of the plasma was only slightly reduced.

(2) Plasma obtained from citrated rabbit blood (1 ml. 3·8 % sodium citrate/10 ml. blood) prepared in an identical manner revealed a constrictor activity equivalent to approximately 0·1–0·5 μg histamine base/ml. of plasma ($n=6$), and this activity disappeared almost completely in the presence of mepyramine.

(3) In the presence of mepyramine the remaining activity of heparin-plasma assayed against 5-hydroxytryptamine creatinine sulphate gave a value approximately 0·6 μg/ml. of plasma.

The possible presence of hydroxytryptamine in heparinized blood but not in citrated blood following antigen-antibody reaction has been previously reported by Humphrey & Jaques (1952).

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The infused living cat. By F. HOWARTH

Total expenditure of energy by adult non-pregnant female rats.

By S. D. MORRISON. *Institute of Physiology, University of Glasgow*

The total 24 hr energy expenditure of eight adult female hooded rats of 100–250 g body weight was measured for sixteen periods, each of from 2 to 5 days. The energy expenditure was measured by closed-circuit respiration calorimetry, using a modification of the apparatus described by Dewar & Newton (1948). During the periods of study the rat's living space, although restricted, permitted nearly normal activity, and free access was allowed to a stock diet.

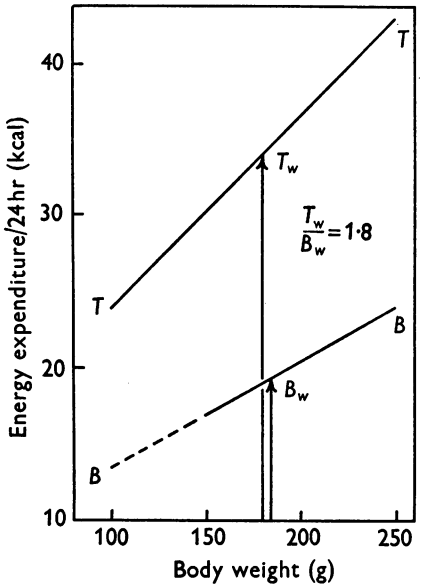

Fig. 1. Line *T*–*T* is total metabolic rate, line *B*–*B* is basal metabolic rate. T_W and B_W are total and basal rates at body weight *W*.

The expenditure of energy varied linearly with body weight over the range studied, according to the equation

$$E = (12{\cdot}54 \pm 1{\cdot}80) + (0{\cdot}121 \pm 0{\cdot}01)\ W,$$

where *E* is energy in kcal and *W* is body weight in g. The line to this equation is shown in Fig. 1.

Benedict (1938) found a linear relationship between body weight and 'basal' metabolic rate of albino rats. His line defining this relation of basal energy expenditure and body weight is also given in Fig. 1. The slopes and locations of these two lines are such that the ratio of total expenditure, under the conditions described above, to the expenditure found by Benedict under basal conditions is, over the relevant range of body weight, essentially constant at 1·8. That is, under the conditions of the present experiment, although the excess of total over basal metabolism increases absolutely with body weight, this excess, as a fraction of the basal expenditure is independent of body weight.

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The part played by changes in arterial p-CO_2 in the production of hyperpnoea in heavy exercise. By R. G. BANNISTER, D. J. C. CUNNINGHAM and C. G. DOUGLAS. *University Laboratory of Physiology, University of Oxford*

Four subjects performed exercise of varying intensity on a motor-driven treadmill. Alveolar air samples were collected by the method of Rahn & Otis (1949), as it seems that these samples probably give a reasonable indication of the changes which occur in the arterial CO_2 tension (Bannister, Cunningham & Douglas, 1953, in preparation). The observed rise in alveolar p-CO_2 above the normal resting level was, of course, quite insufficient to explain of itself the pulmonary ventilation in exercise. There are three possible explanations for this: (1) CO_2 was not a major factor in the exercise hyperpnoea, as many have suggested (e.g. Comroe, 1944), (2) the sensitivity of the respiratory centre to changes of alveolar p-CO_2 (i.e. the response per unit change of stimulus) was increased several fold, (3) the threshold of the respiratory centre to CO_2 (i.e. the level of the alveolar p-CO_2 during natural quiet breathing) was lowered.

There is a simple way of investigating the third possibility. The alveolar p-CO_2 was followed after the exercise in all experiments and minimum values of 30–35 mm Hg were recorded. This depression is caused by the persistence of some degree of hyperpnoea for some time afterwards, probably as a result of the influence of the excess lactate and the higher body temperature which developed during the work. The threshold of the respiratory centre to the CO_2 stimulus has fallen below the normal level because other stimuli, which must be even greater during the exercise, are operative in addition. In experiments designed to test this hypothesis, enough CO_2 was added to the

inspired air during the post-exercise depression to raise the alveolar p-CO_2 to the level found during the preceding exercise. The resulting ventilations were much greater than those produced by the inhalation of the same CO_2 air mixtures before the exercise and were almost comparable to those recorded during the exercise.

If we wish to relate the change in ventilation to the alteration of alveolar (and arterial) p-CO_2, it would be more rational to estimate the latter by comparison not with the normal resting value, but with the reduced threshold which occurs during and after the exercise. When the interference of oxygen lack is avoided by adding oxygen to the inspired air (Bannister, 1953) and the effective p-CO_2 stimulus is estimated in this way, the ventilation and alveolar p-CO_2 in exercise are related in a manner not unlike that found during CO_2 inhalation at rest. This suggests that, when the effects of oxygen lack are absent, the combination of the effective p-CO_2 stimulus and excess lactic acid may be sufficient to explain the hyperpnoea of exercise.

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Further observations on the influence of 5-hydroxytryptamine on bronchial function. By H. HERXHEIMER. *Surgical Unit, University College Hospital Medical School, London*

It has been previously shown that in the guinea-pig 5-hydroxytryptamine (HT), inhaled as 1% aerosol, causes bronchial spasm with severe dyspnoea, followed by convulsions. The picture appears similar to that of histamine or acetylcholine shock. The symptoms are not antagonized by mepyramine (neoantergan) but partly by atropine (Herxheimer, 1953). Lysergic acid diethylamide (LSD), which has recently been found by Gaddum (1953) to antagonize the HT contraction of the rat's uterus, has now been found to antagonize also the HT shock in the guinea-pig. This antagonistic action of LSD (0·2 mg/kg, given intramuscularly) was slightly stronger than that of atropine. The antagonistic action of dihydroergotamine (2 mg/kg) was somewhat weaker than that of atropine. In the anaphylactic microshock of the guinea-pig the same dosages of LSD and dihydroergotamine had no protective effect.

In four normal human subjects the inhalation of 0·67% HT aerosol for 60 sec had no effect on vital capacity or expiratory speed. In three out of six asthmatic patients the same inhalation caused a pronounced asthmatic attack which subsided spontaneously or was abolished by isoprenaline inhalation. In two of the three others who had been free from asthma for a long time, there was a mild reduction of expiratory speed and vital capacity; the third patient

was not influenced. The HT effect in man therefore seems similar to that of histamine and acetylcholine. In the asthmatic patient attacks can be provoked easily by these substances, but normal subjects require much larger amounts to show a mild broncho-obstructor effect.

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The effect of increased venous return upon the heart rate. By J. C. G. Coleridge and R. J. Linden. *Department of Physiology, School of Medicine, Leeds*

Attempts to repeat the experiments of Bainbridge (1915) on the effects of intravenous infusions on the heart rate of the anaesthetized dog have produced conflicting results. To circumvent many of the difficulties attendant on the use of infusions of blood or saline, experiments have been performed on anaesthetized dogs in which the venous return was increased by the gradual opening of a femoral arteriovenous fistula. In the majority of cases the dogs were anaesthetized with a combination of morphia, Dial (Ciba) and sodium pentobarbital (Foltz, Page, Sheldon, Wong, Tuddenham & Weiss, 1950).

Arterial blood pressure was maintained in some experiments by a compensator. Pressures were measured by saline and mercury manometers or by optical manometers. Fistula flows were measured by a rotameter.

If the heart rate was not high (i.e. not more than 120–150), and if sinus arrhythmia was present, then gradual opening of the fistula always produced an increase in rate. This increase was obtained whether right auricular and arterial pressure remained steady, fell or rose. In preparations with a slow heart rate but no sinus arrhythmia no increase in heart rate was obtained.

The use of the compensator and also the results of step-wise lowering of arterial pressure by controlled haemorrhage suggested that arterial baroceptors played no significant part in the increase in rate. Measurements of cardiac output (dye injection method) showed that total venous return was augmented by the amount of the flow through the fistula. External jugular vein flow did not decrease when the fistula was opened if arterial pressure was maintained.

Thermocouples placed in the right auricle showed that the effect was not due to alterations in blood temperature.

Controlled experiments, in which volumes of blood greatly in excess of those involved in pressure compensation passed into the dog, failed to produce an increase in rate.

The results suggest that if the afferent side of the reflex lies on the right side of the heart, pressure, *per se*, is not the adequate stimulus.

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Temperature and humidity of respiratory air. By P. COLE. *Department of Physiology, University of Manchester*

Sampling of air from the respiratory passages indicated that during nasal breathing, inspiratory air in the pharynx was conditioned to within narrow limits over a wide variation in respiratory rate, external temperature and humidity. Air samples obtained from the anterior nares showed a recovery of heat and moisture from expiratory air on its passage outwards from the lungs.

With a subject breathing room air at rates of 8–40 l./min and breathing air of conditions varying from arctic to tropical via a small nasal mask, the inspiratory air in the pharynx was $34 \pm 3°$ C and almost saturated with water. The air-conditioning efficiency of the upper respiratory tract was compared with a simple heat and moisture exchange apparatus, consisting of a glass tube containing moist blotting-paper pellets. With the nose clipped, and the glass tube applied to the lips, a subject breathed so that all respiratory air passed to or from the respiratory tract via this tube. Samples of inspiratory air obtained from the proximal end of the tube (whose only source of heat was expiratory air) were as effectively conditioned as inspiratory pharyngeal air during nasal respiration.

End-expiratory air samples obtained from the pharynx were found to be at body temperature and fully saturated with water. Their condition was considered to be representative of lung air. Expiratory air at the anterior nares was found to be cooler and drier than lung air, about half the heat and water given up by the respiratory tract to inspiratory air being recovered during expiration by cooling and condensation in arctic conditions.

Continuous recording of air temperature in the respiratory passages during the complete respiratory cycle gave reasonable agreement with results obtained from air samples.

On direct connexions between hepatic artery and hepatic veins in the canine liver. By W. H. HORNER ANDREWS, R. HECKER, B. G. MAEGRAITH and H. D. RITCHIE. *Department of Tropical Medicine, Liverpool School of Tropical Medicine*

Translobular arterial branches have been described previously on anatomical grounds (Braus, 1924; Andrews & Maegraith, 1952), and there is strong supporting physiological evidence.

During perfusion of the canine liver, the reaction of the hepatic vein to drugs is greater when injected into the artery than when injected into the portal vein. This is shown especially with substances which are rapidly destroyed in the blood, such as acetylcholine. Addition of eserine to the system decreases the differential effect and carbachol or methacholine (mecholyl), which are not readily affected by cholinesterase, produce comparable effects on the veins, when given into either the artery or the portal vein.

Our hypothesis is that the intra-hepatic courses of the hepatic artery and portal vein are not identical, and that at least a proportion of the arterial blood may pass through the liver more rapidly than portal venous blood.

Five μg of adrenaline injected into the artery usually increases the volume of the liver by constriction of the hepatic veins, although a diminution in arterial inflow occurs; by reducing the amount of adrenaline given it is frequently possible to produce a fall in liver volume, presumably by dilating the hepatic veins. The response to adrenaline given intraportally is a fall in volume, excepting in huge doses. In failing preparations, when the hepatic venous tree is constricting, intra-arterial adrenaline or L-noradrenaline is more effective in producing hepatic venous relaxation (and secondarily increasing the portal inflow) than is injection of the same amount into the portal vein.

It appears that hepatic arterial-hepatic venous connexions are closed by substances which produce arterial constriction; the converse is possibly also true. If 5 μg of acetylcholine is injected into the artery a few seconds before injection of 1–5 μg of adrenaline, the classical responses to both drugs are seen. If, however, adrenaline is injected before acetylcholine, the only response is that to adrenaline. Presumably adrenaline prevents the acetylcholine from reaching the hepatic veins directly. That adrenaline has no direct inhibiting effect on the hepatic veins can be shown by reversing the circulation, with the inflow through the hepatic veins, when responses are obtained to both drugs irrespective of the order of injection.

These results are offered at an early stage of our work for discussion and alternative interpretation.

The expenses were largely defrayed by the Holt Malaria Research Grant.

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Constriction within the canine hepatic venous tree. By W. H. Horner Andrews, R. Hecker, B. G. Maegraith and H. D. Ritchie.
Department of Tropical Medicine, Liverpool School of Tropical Medicine

Since the work of Bauer, Dale, Poulsson & Richards (1932) it has generally been accepted that contraction of smooth muscle around the ostia of the hepatic veins in dogs is mainly responsible for the obstruction to outflow of blood after administration of histamine or in anaphylactic shock, despite evidence that the whole vein may constrict strongly (Weil, 1917; Maegraith, Andrews & Wenyon, 1949).

Three dogs were sensitized to horse serum, and 3–4 weeks later their livers were perfused. The hepatic venous outflow was collected from two separate sites. The first was from a tributary of the hepatic vein via an incompressible polythene tube which was passed 2–3 cm past its ostium and which filled its lumen completely. The remainder of the outflow was taken from the inferior vena cava itself near the caval ostia of the hepatic veins. Both outflows were recorded separately. Injection of histamine and acetylcholine in these livers and in controls led to a comparable reduction in both outflows. Addition of antigen to the perfusion circuit in the case of the sensitized livers almost stopped both outflows, and the subsequent administration of mepyramine malate increased the outflow rate in both.

Another liver was perfused in the reverse direction, the inflow being through two tubes similarly placed as in the experiments outlined above. The artery was clamped and the outflow was via the portal vein. Injection of acetylcholine and histamine into the small tube leading past an ostium slowed the inflow in this tube only. Injection into the main hepatic venous inflow decreased this flow, but the inflow through the smaller tube was unaffected.

It is difficult, therefore, to accept the postulate that the muscle around the hepatic ostia is mainly responsible for producing obstruction to blood outflow, and the hepatic vein of the canine liver may not prove as unique as is accepted at present.

The expenses were largely defrayed by the John Holt Malaria Research Grant. Messrs May and Baker Ltd. donated the mepyramine malate.

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The expenditure of energy and the consumption of food by miners and by clerks, East Fife, Scotland. By J. V. G. DURNIN, R. C. GARRY, R. PASSMORE and G. M. WARNOCK. *Institute of Physiology, University of Glasgow, Department of Physiology, University of Edinburgh, and Ministry of Food, London*

In August 1952, a survey was made of the expenditure of energy and the consumption of food by miners and by clerks in East Fife, Scotland. The method used to find the expenditure of energy was to obtain a detailed and continuous time study throughout the work period and the rest of the 24 hr, to break this down into times spent in different types of activity, e.g. walking, shovelling, hewing, sitting, lying, etc., and then to find the actual 'Calorie value' (in Calories per minute) of each of these activities. The 'Calorie value' of an activity was measured by indirect calorimetry using the Kofranyi-Michaelis respirometer. The total energy expenditure for the 24 hr was then estimated by multiplying the time spent in each activity by its 'Calorie value' and adding all these totals together. In this way nineteen miners and ten clerks were studied each for a period of 1 week. During this week, each of these men also had their consumption of food measured. The dietary survey was carried out by the individual inventory method. All of the food taken by each man was weighed or measured during the 7 consecutive days. The intake of energy in the form of food was calculated by reference to tables of food values, and the intake of protein, of fat, of carbohydrate, of minerals and of vitamins was derived in similar fashion.

The mean daily expenditure of energy was 3660 Cal for the miners and 2800 Cal for the clerks. The mean daily intake of energy was 4030 Cal for the miners and 3040 Cal for the clerks.

Excretion of glucose by the cat's kidney. By M. GRACE EGGLETON and S. SHUSTER. *Department of Physiology, University College, London*

When the blood-sugar concentration is increased, reabsorption of the filtered glucose by the renal tubule cells increases until a maximal value (*Tm*) is attained, in both man and dog. If the filtered load is further increased, *Tm* value remains unchanged.

In the cat, no *Tm* value can be established. The amount reabsorbed continues to increase indefinitely with increasing load, but at a diminishing rate; and, at any given value of the load, becomes smaller as the plasma concentration becomes greater. In Fig. 1 are plotted results obtained in thirty-one experiments in which the plasma-glucose concentration varied between the normal value

(100–250 mg/100 ml.) and 1·05%. At concentrations less than 300 mg/100 ml. (marked +), reabsorption is practically complete up to the highest filtered load encountered. With increasing concentration, the percentage reabsorption at any filtered load is increasingly depressed.

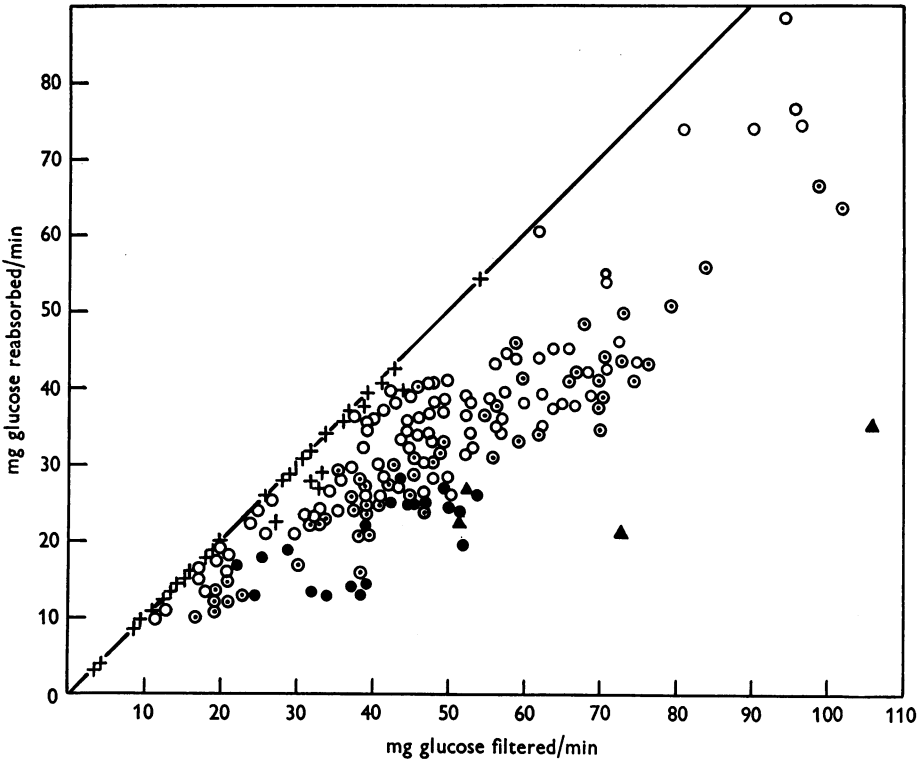

Fig. 1. The effect of increasing plasma glucose concentration on the proportion of filtered glucose reabsorbed. +, plasma glucose concentration <300 mg/100 ml.; ○, plasma glucose concentration 300–500 mg/100 ml.; ⊙, plasma glucose concentration 500–700 mg/100 ml.; ●, plasma glucose concentration 700–900 mg/100 ml.; ▲, plasma glucose concentration >900 mg/100 ml.

If a *Tm* value does in fact exist, it is considerably higher than that obtaining in man and dog (200–250 mg/100 ml. G.F.R.), for rates of reabsorption of 500–600 mg/100 ml. G.F.R. have been observed; and a hypothetical *Tm* value of this magnitude cannot be demonstrated owing to the depressant effect of high plasma-glucose concentration.

Simultaneous observations on urine flow and uterine activity in the conscious bitch. By V. C. ABRAHAMS and MARY PICKFORD. *Department of Physiology, The University, Edinburgh*

Our aim was to discover whether the oxytocic and vasopressor factors of the posterior lobe of the pituitary were simultaneously released from the gland under various physiological conditions which would appear, functionally, to require only the release of one of them. Harris (1947) has shown that electrical stimulation of the hypothalamus in the conscious rabbit causes the release of both factors. Verney (1947) has shown that, amongst other things, a small increase in the tonicity of the blood passing to the central nervous system, and emotion, cause antidiuresis. Our observations were made on ovariectomized bitches in which one carotid had been exteriorized, and in which the upper end of one uterine horn had been brought out to the flank. Activity of the uterus was recorded by inserting in it a balloon filled with normal saline at a pressure of about 30 mm Hg.

It was found that emotional stimuli, and also intracarotid or intravenous injections of concentrated NaCl solution, caused not only an inhibition of water diuresis, but a simultaneous and coterminous increase in uterine activity. The antidiuretic effect could be matched by the intravenous injection of, for example, 3 mU Pitressin, but an equivalent increase in uterine activity needed 80–100 mU Pitocin. The simultaneous injection of 3 mU Pitressin and 5 mU Pitocin did not increase uterine activity observably more than 5 mU Pitocin alone. One bitch was subjected at operation to section of the supraoptico-hypophysial tracts. The day before operation the uterus showed its usual activity. The day after operation the activity was greatly reduced, and during the remaining days of the preliminary polyuria it showed scarcely any spontaneous contractions, though it responded to an intravenous injection of Pitocin. During the normal interphase the uterus showed almost normal contractions. With the onset of the permanent polyuria it again became quiescent. For a time after operation both oral and intravascular NaCl induced uterine contractions, without altering the rate of urine flow.

So far, it appears that in the bitch, the oxytocic and vasopressor factors of the posterior lobe of the pituitary are released simultaneously from the gland, and that the effect produced depends on the sensitivity of the peripheral organ.

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Urinary excretion of antidiuretic hormone during dehydration in rats. By S. E. DICKER. *Department of Pharmacology, University College, London*

The purpose of the present investigation was to re-examine Gilman & Goodman's (1937) claim that the urine of dehydrated rats exerted an antidiuretic effect when injected subcutaneously into test animals, and that the maximum antidiuretic activity in their urine was observed after 48 hr of dehydration. Male albino rats of about 250 g were deprived of water for up to 96 hr. Urine collected in 0·25% acetic acid in saline was *diluted* until isosmotic with blood, and injected intravenously into rats maintained on a constant water load (Boura & Dicker, 1953). Antidiuretic activity was assayed by a four-point method (Dicker, 1953) and expressed in terms of Pitressin (Parke, Davis and Co.). It will be seen from Table 1 that the antidiuretic activity of urine continued to increase substantially after 48 hr of dehydration. The antidiuretic activity in the urine could be abolished, as can that of pitressin, by treating the urine with either 2 N-NaOH or thioglycollic acid (Ames & van Dyke, 1951).

TABLE 1. Amount of antidiuretic activity in urine of dehydrated rats

	Duration of dehydration (hr)			
	24	48	72	96
μU/100 g rat/24 hr	406 (16)	590 (20)	830 (16)	1260 (16)

μU = micro-unit. In parentheses: number of animals.

In normal rats injected with exogenous vasopressin (Pitressin) antidiuretic activity equivalent to about 10% of the injected dose can be recovered from their urine (Dicker, 1953; Ginsburg & Heller, 1953). To see whether the same proportionate excretion is present in dehydrated animals, sixteen rats, chosen at random, were deprived of drinking water and injected subcutaneously twice a day with 1·5 mU/100 g Pitressin, and their urine was assayed for its antidiuretic titre. The amount of exogenous vasopressin was calculated as the difference between the amount of antidiuretic activity found and the mean value of the antidiuretic activity of urine of control dehydrated rats. The amounts of exogenous vasopressin recovered from the urine (from the 1st to the 4th day of dehydration) were 12, 25, 55 and 74% of the amounts injected. Assuming that both exogenous and endogenous vasopressin were excreted in equal proportions by the kidneys it can be calculated that the rate of release of the antidiuretic hormone by the pituitary gland would be constant at 4·0 mU/100 g/24 hr, representing a total of only 16 mU/100 g in 4 days. Preliminary estimates of the antidiuretic-vasopressor activity of the pituitary glands of dehydrated rats have indicated, however, that the degree of depletion of the glands was much greater than that expected from the loss as estimated through urinary excretion.

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Observations on the renal response to motionless standing. By T. M. CHALMERS and R. D. SQUIRES. *Medical Unit, Royal Infirmary, Cardiff, and Chemical Section of the Department of Medicine, University of Pennsylvania Hospital, Philadelphia*

It was previously reported that in recumbent subjects venous congestion of the legs with pneumatic cuffs reduced glomerular filtration rate, renal blood flow and sodium excretion (Chalmers, Lewis & Pawan, 1952). Some effects of motionless standing have now been reinvestigated in normal subjects during water diuresis, urine samples being collected without catheterization at 15 min intervals. The preliminary recumbent period lasted $1\frac{1}{2}$–$2\frac{1}{2}$ hr. After standing still for an hour the mean changes in six subjects (expressed as percentages of the last recumbent values) were: total plasma volume, $11{\cdot}5 \pm 2{\cdot}0$; urine volume, $43{\cdot}8 \pm 5{\cdot}2$; creatinine output, $29{\cdot}5 \pm 5{\cdot}1$; sodium output, $76{\cdot}7 \pm 7{\cdot}1$. The excretion of chloride and potassium also diminished. All values returned towards control levels when the subject lay down again, but recovery of sodium output was delayed relative to that of creatinine.

Bandaging the legs with elastic (Esmarch's) bandages prevented these changes.

Three patients with Addison's disease were studied. When desoxycorticosterone alone was given there was a tendency to fainting with marked reduction in creatinine output and evidence of antidiuretic hormone release. In two experiments where cortisone was also given the renal response to standing resembled that seen in normal subjects.

No increase in the output of sodium (above horizontal control levels) was observed when hydrated subjects were tilted into a 15–45° head-down position for an hour.

Thus pooling of blood in the legs leads to a reduction in sodium excretion which is not wholly accounted for by reduced glomerular filtration, but does not depend on increased adrenal cortical activity. The negative results of head-down tilting are interpreted as evidence against the participation of intracranial 'volume receptors' in sodium regulation (Viar, Oliver, Eisenberg, Lombardo, Willis & Harrison, 1951).

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Changes in the hormone output of the adrenal cortex of the young calf. By W. E. BALFOUR. *Physiological Laboratory, University of Cambridge*

The adult ruminant is resistant to the hypoglycaemic action of insulin (Reid, 1951). The new-born calf behaves in a similar manner for the first 24 hr, but it then becomes very sensitive to insulin. This sensitivity decreases when the animal becomes about thirty days old (Comline *et al.* unpublished observations). During the sensitive period the hypoglycaemic effects of insulin could be largely prevented by the administration of adrenal cortical extract ('Eschatin', Parke-Davis). 11-dehydro-17-OH-corticosterone in equivalent or much larger amounts (liver glycogen deposition assay) had no effect.

Accordingly, the nature and quantity of the hormones secreted by the adrenal cortex have been investigated by a chromatographic method (Bush, 1952) in calves of different ages. In animals less than 2 days old the major components were two members of the 'amorphous fraction' and 17-OH-corticosterone. Thereafter the amorphous fraction was no longer a major component while 17-OH-corticosterone was still present. Corticosterone appeared at about 10 days after birth. In foetal lambs the major component was also a member of the 'amorphous fraction'.

The volume of blood leaving the left adrenal gland of calves either after decerebration and hypophysectomy or during chloralose anaesthesia was measured by a drop counter and returned to the animal. Although the initial flow was high (approximately 40 ml./min/25 kg animal) in anaesthetized animals, only 10–20 ml./min was recorded in decerebrate preparations. The rate of flow declined to less than 5 ml./min by the end of the first hour for the decerebrate, and by the end of the third hour for the anaesthetized preparations. The intravenous infusion of small amounts of adrenocorticotrophic hormone (ACTH) (Organon, Armour) caused a temporary increase of blood flow from the gland; this could be repeated with further doses of ACTH. This response to ACTH was always obtained in calves from 10 to 40 days of age, but was either very small or not appreciable in animals less than 8 days old.

The hormones were separated from the adrenal effluent blood and estimated chemically as formaldehydeogenic steroids (Daughaday, Jaffeé & Williams, 1948), and after reaction with 2:4-dinitrophenylhydrazine (Gornall & Macdonald, 1953). When ACTH was given to calves less than 8 days old there was

no increase in steroid output. The effect of ACTH in calves from 8 to 40 days of age was not to increase the concentration of hormones in the blood but only to increase the amount of blood leaving the gland.

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The actions of some derivatives of adrenaline and noradrenaline on the nictitating membrane and the heart rate of the cat.

By I. R. Innes and H. W. Kosterlitz. *Physiology Department, University of Aberdeen*

Chronic preganglionic denervation ('decentralization') uniformly increased the response of the nictitating membrane to all sympathomimetic substances examined so far. When the responses of postganglionically denervated membranes ('denervation') were compared with 'decentralized' membranes, the results varied considerably (Table 1). The presence of a hydroxyl group in position 3 of the benzene ring favoured potentiation by 'denervation' while in its absence 'denervated' and 'decentralized' membranes showed similar responses or, as in the case of tyramine, the response of the 'denervated' membrane was depressed.

Intravenous injection of 2–3 mg/kg of cocaine hydrochloride altered the responses of the 'decentralized' membranes so as to make them similar to those of the 'denervated' membranes. In the acutely denervated heart, on the other hand, cocaine potentiated the response to noradrenaline but not to any other of the substances given in Table 1.

Table 1. Responses of 'denervated' nictitating membranes compared with those of 'decentralized' membranes

1-(3, 4-dihydroxyphenyl)-2-methylaminoethanol (adrenaline)	0 to +
1-(3, 4-dihydroxyphenyl)-2-aminoethanol (noradrenaline)	+ +
1-(3-hydroxyphenyl)-2-methylaminoethanol (neosynephrine)	+ to + +
1-(3-hydroxyphenyl)-2-aminoethanol	+ + +
1-(4-hydroxyphenyl)-2-methylaminoethanol (sympatol)	0
1-(4-hydroxyphenyl)-2-aminoethanol	0 to −
1-(4-hydroxyphenyl)-2-aminoethane (tyramine)	− −

While the results seem to suggest that the sensitization of the 'decentralized' nictitating membrane may be due to a single cause, perhaps the inhibition of amine oxidase, the effects of 'denervation' and of cocaine cannot be explained in this manner. First, while noradrenaline and tyramine are both

rapidly acted upon by amine oxidase, 'denervation' or cocaine potentiates the action of noradrenaline and depresses that of tyramine. Secondly, cocaine potentiates the response of the nictitating membrane but not the chronotropic response of the heart to adrenaline and noradrenaline derivatives not possessing a hydroxyl group in position 4. The changes brought about in the effectors by 'denervation' cannot be attributed to the degeneration of nerve fibres as such, as cocaine produces the same effect in doses which do not abolish nerve conduction.

The effects of penicillin on the weight gained by kittens. By CECILIA D. DICKINSON and PATRICIA P. SCOTT. *Department of Physiology, Royal Free Hospital School of Medicine, London, W.C.* 1

Saunders (personal communication) found that the addition of 66 mg procaine penicillin/kg to his basic diet (1953) produced a somewhat variable increase in the weight gained by kittens compared with control periods when penicillin was omitted from their diet.

Two preliminary experiments indicated that the addition of procaine penicillin to a high protein diet gave an increase in the weight gained by kittens aged between 14 and 22 weeks. Twelve kittens from four litters were used. The amounts of procaine penicillin (1 mg = 1000 i.u.) added were 15, 30 and 60 mg/kg of diet (wet weight). At all these levels some increase in the weight gained was apparent when compared with litter-mate control kittens receiving the basic diet alone. A noticeable decline in the weight gained was observed in the week immediately following the withdrawal of the penicillin supplement, followed by a resumption of a normal increase in weight. 30 mg/kg appeared to be a suitable level of supplementation and was used in subsequent experiments.

Twenty-four kittens from seven litters were weaned on to the basic diet at approximately 6 weeks of age. They were divided into 2 groups whose mean weights differed by only 6 g. After 14 days, the mean weight of the kittens receiving penicillin had increased by more than 200 g over the mean weight of the kittens not receiving penicillin. Moreover, the kittens receiving penicillin appeared to be healthier and livelier than those in the control group, some of which had to be temporarily isolated for low-grade respiratory infections.

We are grateful to the Royal Society and to the Medical Research Council for grants which enabled this work to be undertaken, and also to Glaxo Laboratories Ltd. and Chappie Ltd. for materials.

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Release of histamine by tryptamine and 5-hydroxytryptamine.

By W. FELDBERG and A. N. SMITH. *National Institute for Medical Research, Mill Hill, N.W. 7*

Tryptamine and 5-hydroxytryptamine have the ability to release histamine from the living tissue. This finding brings them into line with the large group of amines having this property (MacIntosh & Paton, 1949). The histamine releasing activity of these compounds, which is somewhat greater than that of propamidine and about 100 times less than that of 48/80, was observed under the following conditions.

(*a*) After arterial injection of either tryptamine or 5-hydroxytryptamine into the perfused skin flap or gastrocnemius muscle of the cat.

(*b*) After arterial injection of tryptamine into the perfused skin flap of the dog.

(*c*) From rat's skin, at the site of a subcutaneous injection of tryptamine and from rat's skin and skeletal muscle after repeated intraperitoneal injections.

In the experiments (*a*) and (*b*) the released histamine was assayed in the venous perfusate; in the experiments (*c*) the release was shown by determining the reduction in tissue histamine.

The assay of histamine on the atropinized guinea-pig's ileum was rendered difficult by contamination of the perfusate with large amounts of either tryptamine or 5-hydroxytryptamine respectively. To overcome this difficulty, the perfusate had first to be assayed for these substances on the atropinized rat's colon. They were then added in appropriate amounts to the solutions of histamine used for assaying the perfusate on the guinea-pig's ileum.

Compound 48/80, D-tubocurarine, propamidine and tryptamine, when added to the bath in which the guinea-pig's ileum is suspended, produce periods of increased rhythmicity and tone. This effect may be preceded with some of these histamine liberators by an initial, strong contraction. It was examined whether the increased rhythmicity and tone resulted from a slow and continuous release of histamine from the intestinal wall, and it was found that after 48/80, and to a smaller extent after the other histamine liberators, a substance acting like histamine and probably identical with it could be detected in the bath fluid collected during the periods of increased rhythmicity and tone, suggesting that these motor effects were, at least in part, the outcome of histamine release from the intestinal wall. The finding that mepyramine greatly reduced the tone and diminished but slightly the rhythmic activity could be explained on the assumption that it antagonizes only histamine which has diffused out into the surrounding fluid, but not at the site of release.

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Reactions of nerve-free and chronically denervated plain muscle to drugs. By D. H. L. EVANS and H. O. SCHILD. *Departments of Anatomy and Pharmacology, University College, London*

In a previous communication (Evans & Schild, 1953) we described the effect of acetylcholine, barium, eserine and nicotine on intestinal circular muscle of the cat immediately after removal of the enteric nerve plexuses. The present experiments deal with the drug responses of (*a*) cat circular muscle freed of the myenteric plexus of Auerbach *in vivo*, the animals being allowed to survive for periods of 13–27 days in order to allow degeneration of the intramuscular nerve endings of the plexus; (*b*) the amniotic membrane of 10- to 12-day incubated chicks, which is known to contain plain muscle entirely devoid of ganglion cells and nerve fibres. The following is a summary of our findings:

(1) 5-*hydroxytryptamine* (*serotonin creatinine sulphate*) produces powerful rhythmic contraction of the amnion. Since the amnion is nerve-free this action cannot be due to release of acetylcholine from nerve endings, but suggests a direct action on plain muscle.

(2) *Nicotine* produces inhibition of spontaneous contractions and lowering of tone in the amnion. It produces both stimulation and strong inhibition in denervated cat circular muscle, both effects being antagonized by hexamethonium. These results suggest that nicotine has a residual action on nerve endings or plain muscle which can be antagonized by hexamethonium.

(3) *Eserine* produces powerful rhythmic contractions in the amnion and occasionally in denervated circular muscle. These effects may well be due to some non-nervous acetylcholine present in the muscle since a small dose of acetylcholine added to a subthreshold dose of eserine produces similar rhythmic effects.

(4) Both preparations are stimulated by *acetylcholine*. Comparison of the acetylcholine threshold of circular muscle freed of Auerbach's plexus with normal muscle revealed no diminution in threshold after denervation.

(5) Both preparations are inhibited by *adrenaline* and by *isoprenaline*, the latter being more active.

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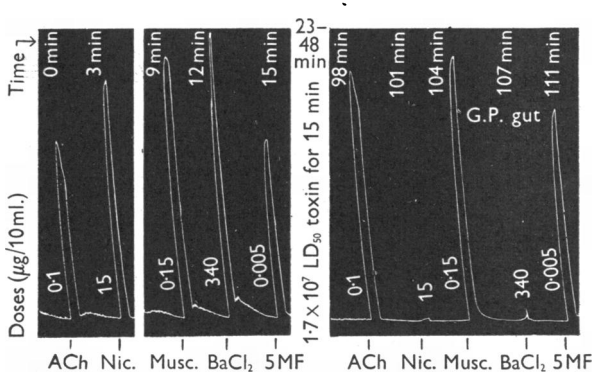
***In vitro* effects of Botulinum toxin (type D).** By N. AMBACHE and A. W. LESSIN. *Ophthalmological Research Unit* (*Medical Research Council*), *Institute of Ophthalmology, Judd Street, London, W.C.* 1

Guinea-pig (G.P.) and rabbit (R.) ilea have been exposed *in vitro* to $1–2 \times 10^7$ mouse LD_{50} (in a 10 ml. bath) of a purified Botulinum type D toxin. Even after short exposures of 5–20 min there is pharmacological evidence of (post-

ganglionic) motor neurone paralysis in the gut, becoming total within ½–1 hr and persisting to the end of the experiment. This procedure provides a rapid method of irreversible motor 'denervation' and allows a clear distinction to be made between drugs which act primarily on the motor neurones (group A) and those which act principally on the muscle fibres (B).



Group A. The action of previously stimulant doses of nicotine, *m*-bromophenyl ether of choline, 1, 1-dimethyl-4-phenyl-piperazinium (DMPP) is abolished.

Group B. Responses to ACh, Muscarine, 2268 F and 5-Me-furfuryltri-Me-NH_4 (5 MF) are not significantly decreased.

Group C (mixed). The effect of certain doses of barium is either abolished (G.P.; see figure) or reduced. KCl is also reduced.

Group D. The spastic effect of eserine is reduced (R.)

We are indebted to Drs M. Sterne and L. M. Wentzel (Pretoria) for a gift of toxin and to Dr G. Chen (Detroit) for the DMPP.