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COMMUNICATIONS

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The effect of oestrogenic or progestogenic substances on a modified bromsulphthalein test

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Tindall & Beazley (1965) used the modified bromsulphthalein test devised by Richards, Tindall & Young (1959) to show alterations in liver function in normal pregnancy. They thought these changes were caused by the raised level of circulating oestrogens. Others, reporting alterations in liver function in women taking oral contraceptives, have suggested that the changes reflect an oestrogenic rather than a progestogenic influence (Adlercreutz & Ikonen, 1964).

Using the modified bromsulphthalein test two groups of puerperal women were compared, one taking 17 alpha acetoxy 6-methyl progesterone for suppression of lactation and one taking diethylstilboestrol or ethinyloestradiol. The dose of diethylstilboestrol was 10 mg three times a day for 3 days then 10 mg twice a day for 3 days. The dose of progesterone was the same. Those women taking ethinyloestradiol received 2 mg for 1 day then 1 mg daily. The tests were performed on the seventh post-partum morning. The dose of bromsulphthalein was 300 mg given by intravenous injection. Controls were taken from women breast-feeding or women who, although bottle-feeding, were on no therapy for suppression of lactation. In addition, using the same dose of bromsulphthalein, a group of normal pre-menopausal women were tested.

A comparison of the constants obtained is shown in tabular form. The rates of transfer of bromsulphthalein from one compartment to another are expressed as mg transferred

	Direction of transfer of bromsulphthalein	
	Liver to plasma mean ± S.E. of mean	Liver to bile mean ± S.E. of mean
Pre-menopausal (19)	0·90 (±0·67)	5·96 (±2·48)
Breast feeding (20)	0·99 (±0·57)	4·35 (±2·54)
Stilboestrol and ethinyl oestradiol (22)	1·52 (±0·50)	1·73 (±0·82)
Progesterone (21)	0·93 (±0·30)	3·89 (±1·37)

each minute per 100 mg contained in the compartment from which the transfer is taking place. The number of patients is shown in brackets.

The transfer rate of return of bromsulphthalein from the liver to the plasma is significantly increased ($P<0.01$) and the transfer rate of elimination of bromsulphthalein into the bile is significantly reduced ($P<0.001$) compared with the pre-menopausal, breast feeding and progesterone groups.

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Effect of long-term continuous steroid therapy on adrenal function

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Adrenal function was evaluated in one hundred asthmatic patients receiving continuous steroid therapy for periods ranging from 5 months to 10 years, and in twelve asthmatic patients who have not received steroids. An intravenous infusion containing 40 units of corticotrophin (ACTH) was given over 4 hours and this was followed by intramuscular injections of 40 units of ACTH-gel given at 12-hourly intervals for 3 days. Plasma and urinary 17-hydroxycorticosteroids (17-OH-CS) were measured, using the Porter–Silber reagent, for 2 days before and during adrenal stimulation. Adrenal steroids were not given during the tests.

To evaluate adrenal function, the one hundred patients were divided arbitrarily into three groups according to the maximum urinary 17-OH-CS output achieved during ACTH. Seventy-five patients had satisfactory adrenal function (the values were equal to or over 15·4 mg/day; the minimum response recorded in control subjects); thirteen had partial adrenal suppression (values between 8·5 and 15·4 mg/day), and the remaining twelve had adrenal suppression (values of 8·5 mg/day or less).

In eight patients adrenal suppression could not be reversed despite continuous stimulation with ACTH-gel for 13 days. This finding has important clinical implications. In three of these eight patients, severe and persistent adrenal failure was demonstrated earlier during attacks of status asthmaticus despite treatment with large doses of corticotrophin (El-Shaboury, 1966).

The response to ACTH was delayed even in those patients with satisfactory adrenal function; in 65% the maximum urinary 17-OH-CS output was not achieved until the third day of ACTH, and in 44% subnormal plasma 17-OH-CS values were found during the ACTH infusion but not during the subsequent days of ACTH injections. Adequate

adrenal stimulation is therefore necessary for evaluation of adrenal function in steroid-treated patients.

Adrenal function was not influenced by the age of the patient nor by the duration of asthma. It was significantly influenced ($P<0.01$) by the duration of steroid therapy and by the total amount of steroid received. In only three of twenty-five patients with adrenal suppression or partial adrenal suppression was the duration of therapy less than 5 years, and in only one patient was the total amount of steroid given less than 12 g prednisone.

Although the maximal plasma and urinary 17-OH-CS values during ACTH were highly correlated, the latter were the better index of the effect of steroid therapy on adrenal function.

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The circulatory effects of atropine during anaesthesia

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Circulatory changes following the intravenous injection of atropine sulphate (0·6 mg) were investigated in thirty-five patients undergoing surgery and anaesthesia. The patients were divided into three groups according to the anaesthetic agents and techniques employed.

Group 1: Fifteen patients—nitrous oxide (60%), oxygen, halothane (1–2%). Spontaneous ventilation.

Group 2: Ten patients—nitrous oxide (60%); oxygen, (+)-tubocurarine. Artificial ventilation.

Group 3: Ten patients—nitrous oxide (60%), oxygen, gallamine triethiodide. Artificial ventilation.

All patients received an opiate plus hyoscine or atropine as a premedication.

The principal results are given in Table 1.

TABLE 1

CIRCULATORY CHANGES 2–5 MINUTES AFTER GIVING ATROPINE

The changes are expressed as a percentage of the control value (100%), followed by the probability, *P*, that the changes observed are due to chance.

	Heart rate			Cardiac output (% change)	Stroke volume (% change)	Mean arterial pressure (% change)	Peripheral resistance (% change)
	Initial (beats/min)	Final (beats/min)	(% change)				
Group 1	61	110	+83 $P<0.001$	+48 $P<0.001$	−17 $P<0.01$	+24 $P<0.001$	−17 $P<0.001$
Group 2	68	96	+43 $P<0.001$	+31 $P<0.001$	−6·3 $P>0.9$	+12 $P<0.001$	−14 $P<0.01$
Group 3	97	105	+10 $P<0.01$	+11 $P<0.01$	+2·4 $P>0.8$	+3·3 $P>0.8$	−5·3 $P>0.6$
Mean of all patients	73	102	+51	+33	−8·6	+15	−13

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Berry, Thompson, Miller & McIntosh (1959) showed in conscious volunteers that when sufficient atropine was given to raise the heart rate, the cardiac output also rose, but the stroke volume and central venous pressure fell. When this fall in venous pressure was prevented by infusion of albumin, the stroke volume remained at the control level.

Weissler, Leonard & Warren (1957) found that peripheral venous pooling was associated with a marked fall in stroke volume after giving atropine.

Our patients in Group 1 received halothane which has a generalized sympathetic blocking effect (Price & Price, 1966). This type of anaesthetic is associated with skin vasodilatation and peripheral pooling and this may have been responsible for the fall in stroke volume in this group. Little modification of the effects of atropine occurred in Group 2. In Group 3, because of the higher initial heart rate caused by gallamine, the mean heart rate increase was considerably smaller and the other parameters were correspondingly less affected.

Increase in cardiac output following the injection of atropine depends on the change in heart rate. When the cardiac output rises the arterial pressure also rises and the total peripheral resistance falls. The algebraic sum of the percentage changes in these parameters closely follows the percentage change in cardiac output.

It is concluded that the circulatory responses to the intravenous injection of rate-increasing doses of atropine vary markedly according to the physiological and pharmacological background against which it is exhibited.

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Salicylate and erythrocyte metabolism

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We have previously shown that a significant positive correlation exists between the inhibition of platelet clumping and the increase in whole blood ATP : ADP ratio when aspirin is administered in high doses to man (Davies, Hughes & Tonks, 1968). The mechanism of this change in whole blood adenine nucleotide balance has been studied.

Sodium salicylate in concentrations ranging from 1 to 5×10^{-3} M increases the utilization of glucose and the production of lactate when added either to freshly withdrawn heparinized whole blood or to fresh erythrocytes washed and suspended in Tyrode-Locke solution.

A given concentration of salicylate is less effective in stimulating glucose uptake in whole blood than in erythrocytes suspended in Tyrode-Locke solution. The reasons for this are discussed.

The rate of glycolysis of erythrocytes suspended in potassium phosphate buffer is far in excess of that of erythrocytes from the same donor suspended in Tyrode-Locke solution and incubated simultaneously at 37° C for the same period of time. Salicylate (5×10^{-3} M) produces only a slight increase in glycolysis when erythrocytes are suspended in potassium phosphate buffer compared with the pronounced stimulus of glycolysis in an aliquot of the same cells suspended in Tyrode-Locke solution. The effectiveness of salicylate in stimulating erythrocyte glycolysis is masked in phosphate buffer by the elevated glycolytic rate caused by the unphysiological concentrations of potassium and phosphate in this medium.

Because salicylate has been shown to stimulate ATPase activity in liver mitochondria (Penniall, 1958; Falcone, Mao & Shrago, 1963; Charnock & Opit, 1962) the effect of the drug on erythrocyte ATPase activity has been examined.

Na^+–K^+ dependent transport ATPase (which accounts for a significant proportion of erythrocyte lactate production) can be completely inhibited by ouabain at a concentration of 10^{-5} M (Whittam, Ager & Wiley, 1964). Preliminary results show that ouabain (10^{-5} M) has no effect on the increase in lactate production seen in the presence of 5×10^{-3} M sodium salicylate. This suggests to us that the increase in the rate of erythrocyte glycolysis induced by sodium salicylate is not mediated by stimulation of Na^+–K^+ dependent ATPase.

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Acidification of urine: normal response to ammonium chloride

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Ammonium chloride, since its introduction as an acidifying agent by J. B. S. Haldane, has been used in repeated doses and more recently in a single dose to assess ability to acidify the urine in particular circumstances or in renal disease. Following a single dose of ammonium chloride of 0·1 g (2 m-equiv)/kg body weight swallowed at 10 a.m., Wrong & Davies (1959) found that urine collected hourly from noon to 6 p.m. from eleven people who were considered to have normal renal function had a mean pH of 4·81. The directly observed range of mean urine pH for these individuals was from 4·60 to 5·24. Four were

hospital patients. In retrospect one of them, subject No. 9, convalescing from pneumonia, was abnormal. His plasma total CO_2 was initially 32·3 mM, and his mean urine pH of 5·24 was 0·30 pH units higher than that of any other subject. This must have increased the scatter of observations. The present study is an attempt to define more accurately the response of normal people to this standard dose of ammonium chloride. Larger doses tend to cause nausea; half this dose has given highly variable responses.

Colleagues and students formed the present ten subjects. They were Nos. 2, 3, 4, 6, and 7 of the previous study, four men aged 19, 20, 20 and 27, and one woman aged 20. The test procedure was unchanged except that greater care was taken to ensure that during a test subjects did not exercise, eat large meals or drink excessively, aiming to keep urine flow below 2·5 ml./min.

Overall pH of the hourly collections (60) was (mean, S.D., and S.D. as % of mean) $4·79 \pm 0·17$ (3·5%). Trend with time was insignificant. Most of the variation was between individuals. The data give an upper limit of response of the urine pH, to this standard dose and with these precautions, at $P=0·05$ of 5·13 pH and at $P=0·01$ of 5·23 pH.

Excretion of ammonium showed more variation, being (60) 51 μ-moles/min ± 15 (30%). This variation was not reduced by factoring by subjects' body weight, being then (60) 0·72 μ-moles/kg.min ± 0·22 (31%). In the four subjects in whom it had been measured, factoring by simultaneous excretion of creatinine reduced the variation to give (23) 4·36 μ-moles/μ-mole creatinine per min ± 0·63 (14%), or 38·6 μ-moles/mg creatinine.min ± 5·6. This may allow use of untimed urine collections to assess excretion of ammonium, as well as the acidification of urine.

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"Converting enzyme" activity in plasma

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The physiologically inactive decapeptide angiotensin I is converted to the active octapeptide angiotensin II by the action of "converting enzyme" which removes the terminal dipeptide histidyl-leucine. Radioactive phenylthiocarbamyl-angiotensin I ([^{35}S] PTC-angiotensin I) prepared from synthetic valyl^5-angiotensin I amide in a manner similar to that used for the preparation of [^{35}S] PTC-angiotensin II (Osborn, Louis & Doyle, 1966) was used to study "converting enzyme" activity in plasma of hypertensive and normotensive subjects using physico-chemical techniques similar to those described by Osborn, Jerums & Jupp (1968) for radioactive PTC-angiotensin II. There was no demonstrable difference between the two series in ability to convert PTC-angiotensin I to PTC-angiotensin II. *In vivo* studies in the rat, guinea-pig and rabbit showed that the conversion of angiotensin I to angiotensin II was very rapid. The non-radioactive PTC-derivative of angiotensin I

was also shown to produce a prompt rise in blood pressure in the rat and rabbit but to possess less than the pressor activity expected had the conversion to PTC-angiotensin II taken place as rapidly as that of angiotensin I to angiotensin II. These findings indicate that PTC-angiotensin I is suitable for comparative studies but not for establishing absolute values. Studies using angiotensin I and either human blood or plasma prepared with heparin as anticoagulant and the isolated rat colon (Regoli & Vane, 1964) with both superfusion and organ bath techniques suggested that "converting enzyme" activity was too slow to account for the very rapid conversion observed *in vivo*.

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A comparison of the effect of angiotensin and noradrenaline on proximal and distal tubular diameter and urine flow velocity in the rat kidney

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Angiotensin II exerts a dual effect on the kidney of man, rabbit and rat, and can elicit either diuretic or antidiuretic responses. In the present experiments, the action of angiotensin II was investigated on urine flow rate in the proximal and distal convoluted tubules of the rat, by direct observation of these nephron segments on the kidney surface.

The experimental preparation has been described by Steinhausen (1963). The exposed left kidney of rats was examined by incident-light microscopy and tubular transit times obtained following intravenously injected lissamine green. Measurements of tubular volume were made from microphotographs of the renal surface.

Noradrenaline or angiotensin II (synthetic Val^5 octapeptide) was infused intravenously in a dosage of 0·5 μg/kg.min. This dose usually produced a marked diuretic and natriuretic response in non saline-loaded rats with both substances, although angiotensin occasionally reduced both urine flow and sodium excretion. When diuretic, angiotensin increased proximal tubular volume and greatly increased distal tubular volume. Distal tubular diameter was unchanged in experiments in which angiotensin was antidiuretic. Urine flow velocity was markedly reduced in proximal and distal nephron segments in both diuretic and antidiuretic phases.

Noradrenaline, however, in a dose producing a similar increase in urine flow to that obtained with angiotensin, produced only a slight dilatation of distal tubules and did not increase proximal tubular diameter. Urine flow velocity was unchanged or increased in both segments. Osmotic diuresis, produced by the intravenous injection of 2 ml. of 20% dextrose solution, resulted in an increased distal tubular diameter but did not prolong dye transit times.

The qualitative differences between the actions of angiotensin and noradrenaline on renal tubular transit time and volume might be explained by differential effects of the two substances on the volume delivered to the distal convoluted tubule, or on the resistance to flow in the nephron.

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The abortifacient action of warfarin in cattle

By D. M. PUGH (introduced by C. W. M. WILSON), *Department of Pharmacology, Trinity College, University of Dublin, Eire*

On very limited experimental evidence the estimated dose of warfarin required to produce a 50% mortality in cattle is placed at 200 mg/kg daily for 5 days (Papworth, 1958). This high dosage is the basis of the belief that ruminants are resistant to the action of warfarin, and this figure contributes to the widely publicized claim that warfarin is not dangerous to domestic animals.

Following the ingestion of a mean daily dose of 17·6 g of a warfarin-containing rat-bait for 10 days, it was observed that eight out of twenty-three pregnant cattle aborted within the following 2 days. This amount of rat-bait would have yielded a daily dose of warfarin in the range of 40–120 mg/cow. Assuming an average cow weight of 400 kg, this would have represented a dose rate of 0·1–0·3 mg/kg.

This demonstration of an abortifacient action threw considerable doubt on the safety of warfarin and prompted a re-investigation of its toxicity for cattle. Four Swiss Brown cattle received warfarin orally at a daily dose rate of 0·25 mg/kg. After 10 days the mean prothrombin time had fallen below 20% of normal. This suggests that the currently accepted estimated dose required to produce a 50% mortality in cattle is too high and indicates the need for further investigation.

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Absorption, distribution and excretion of (2-^{14}C) amiphenazole hydrochloride in female rats

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There is little information available regarding the fate in the body of the respiratory stimulant, amiphenazole hydrochloride. Such knowledge may be helpful in explaining

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the difference in clinical effectiveness of the drug when it is given by oral and intravenous routes to patients suffering from chronic respiratory failure (Hughes, 1963; Nelson & Wallace, 1965).

In female albino rats (90–110 g, A. Tuck & Son, Essex), absorption of (2-^{14}C) amiphenazole hydrochloride (4 μc/mg in a dose of 10 mg/kg) from the gastrointestinal tract occurs almost exclusively in the small intestine; 90–95% of the radioactivity administered is absorbed in 1 hr. When the drug is administered by either intraduodenal or intravenous injection, approximately 10% of the label appears in the bile within 4 hr. Some of this material is then reabsorbed. Radioactivity rapidly appears in the urine, 60% of an oral dose and 70% of an intravenous dose being excreted in 6 hr. In 24 hr 80–85% of the radioactivity is recovered in the urine and faeces.

Significant levels of radiocarbon are found in the brain, liver, kidney, skeletal muscle, adrenal gland and gastrointestinal tract soon after administration of the labelled drug either orally or intravenously. The level of radioactivity in the blood is very low at all times after administration of the compound by either of these routes and low levels of activity are found also in body fat, the skin and bone. The maximum level of radioactivity in the brain is reached much sooner after intravenous administration than after oral administration. When identical doses (10 mg/kg) are given, this level is twice as great after intravenous than after oral dosing and these findings may explain why amiphenazole is more effective when administered intravenously. From the results of chromatography of methanolic extracts of brain from female rats receiving ^{14}C-amiphenazole, it is concluded that the drug itself and not a metabolite is responsible for its pharmacological actions in the central nervous system.

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An effect of adrenalectomy upon catecholamine metabolism

By V. M. AVAKIAN and B. A. CALLINGHAM*, *Department of Pharmacology, University of Cambridge*

The cardiac noradrenaline stores of adrenalectomized rats have been shown to be more sensitive to depletion by β-tetrahydronaphthylamine than those of normal rats although there is little or no change in endogenous content (Avakian & Vogt, 1966). This effect could be brought about by impairment of the normal uptake processes involved in the recapture of released amine.

The uptake of ^{3}H-noradrenaline and ^{3}H-adrenaline has been studied using isolated perfused hearts from adrenalectomized and normal male and female rats. Adrenalectomized animals were maintained on 0·9% sodium chloride solution and those which received, subsequent to operation, daily injections of hydrocortisone (10 mg/kg) had the

choice of either sodium chloride solution or water. A perfusion concentration of 10 ng/ml. of either noradrenaline or adrenaline was used to study the uptake process which operates at low concentration of catecholamines ($Uptake_1$; see Iversen, 1967), and of 5 μg/ml. for the process which operates at high concentrations ($Uptake_2$; see Iversen, 1967).

No significant difference could be found between the hearts of adrenalectomized and control rats in their ability to take up noradrenaline or adrenaline perfused at 10 ng/ml. When the amines were perfused at 5 μg/ml., however, there was a significant reduction in uptake of both noradrenaline and adrenaline by the hearts of adrenalectomized rats. This effect was present at 3, 7 and 14 days following operation and could be completely prevented by daily treatment with hydrocortisone (10 mg/kg). Simultaneous determination of the catecholamine metabolites formed in the hearts showed that there was a 30% rise in the proportion of deaminated metabolites following adrenalectomy, although the total uptake was reduced. Because most of the catecholamine taken up by the $Uptake_2$ process appears not in the granular fraction, but in the supernatant following high speed centrifugation, it is possible that the reduced uptake and increased metabolism is the result of an increase in the content of monoamine oxidase (MAO) or increased penetration of the substrate to the enzyme.

Measurement of the MAO activity of homogenates of heart tissues using ^{3}H-tyramine as substrate showed 2–5 fold increases following adrenalectomy. No changes in catechol-O-methyltransferase were observed. Treatment with hydrocortisone reduced the MAO activity following adrenalectomy to that of the controls. Almost complete inhibition of MAO activity produced by two doses of pheniprazine (10 mg/kg) significantly antagonized the reduction in uptake but did not restore it to control level.

It is suggested that the reduction in the uptake of catecholamine by the hearts of adrenalectomized rats results, at least in part, from an increase in the ability of the MAO in the tissue to metabolize the accumulated amine.

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Vascular sensitivity following adrenalectomy in the rat

By F. J. IMMS* and M. T. JONES (introduced by H. BARCROFT), *Sherrington School of Physiology, St. Thomas's Hospital Medical School, London, S.E.*1

Adrenalectomized or sham-adrenalectomized male rats of the Wistar strain weighing 200–400 g were anaesthetized with pentobarbitone sodium (60 mg/kg I.P.). The blood pressure was recorded with a mercury manometer from a carotid artery and a femoral vein was cannulated for injection.

It was confirmed that adrenalectomized animals had a lower blood pressure. There was a marked decrease in pressor response to both noradrenaline and vasopressin.

Sensitivity was similar in adrenalectomized animals maintained on 0·9% NaCl solution and in those given tap water to drink.

It is suggested that the loss of sensitivity in the adrenalectomized rat results from the absence of the "permissive action" (Ingle, 1952), and the lower blood pressure results from insensitivity of the cardiovascular system to sympathetic stimulation. It has previously been shown that adrenalectomized animals excrete large amounts of catecholamines in the urine—an effect which can be prevented by treatment with physiological doses of corticosterone (Imms & Jones, 1967). This catecholamine secretion may represent reflex overactivity of the sympathetic nervous system in an attempt to raise the blood pressure to normal.

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The action of prostaglandin E_1 on the guinea-pig isolated intestine

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Prostaglandin E_1 contracts segments of isolated intestine. An attempt has been made in these experiments to analyse this action of E_1 on the isolated small intestine of the guinea-pig.

Contractions of the isolated intestine induced by doses of E_1 ranging from 1·5 to 40 ng/ml. were measured and the dose-response curves drawn. Such curves were constructed from experiments in which the tissue was stimulated by E_1 in the presence of increasing concentrations of atropine (0·01 to 0·1 μg/ml.), in the presence of diisopropylfluorophosphate (5 μg/ml.), and in the presence of procaine hydrochloride (5 μg/ml.). Both atropine and procaine reduced the responses of the intestine to E_1 but they did not abolish them. Diisopropylfluorophosphate caused some potentiation of the responses of the intestine to E_1. Control segments of intestine to which none of these three compounds were added showed little change in their responses to E_1. These results imply two sites of action of E_1 on the guinea-pig intestine, one involving the intramural nerve plexuses and the other involving the smooth muscle cells.

The responses of the intestine to E_1 were reduced by hexamethonium (50 μg/ml.); this reduction was less than that observed in experiments when the intestine was stimulated with nicotine. Thus, E_1 may stimulate pre-ganglionic fibres, or cholinergic receptors on the ganglion cells or may affect the transmitter released from the post-ganglionic nerve fibres.

Transmural stimulation of the guinea-pig isolated intestine with a pulse duration of 0·4 msec stimulates only the intrinsic nerve plexuses (Harry, 1962). E_1 (2·0 ng/ml.) caused an increase in the maximal and non-maximal twitch height of the intestine produced

by transmural stimulation. Furthermore a dose of E_1, which had no stimulant effect when the intestine was not electrically stimulated, caused an increase in the tone of the tissue when it was electrically stimulated.

E_1 can potentiate the spasmogenic action of various substances on the guinea-pig uterus (Clegg, Hall & Pickles, 1966). On the isolated intestine E_1 (0·5 to 8 ng/ml.) potentiated the responses induced by acetylcholine over a whole dose range (1·25 to 40 ng/ml.).

Transmural stimulation of the isolated intestine releases acetylcholine from post-ganglionic fibres to produce a twitch response of the smooth muscle. E_1 may potentiate the action of released acetylcholine to produce its increase in this twitch response. Furthermore the action of E_1 involving the intramural nerve plexuses on the isolated intestine may be by potentiation of endogenously released acetylcholine.

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Investigation of the spasmogenic effect of manganese on the guinea-pig isolated ileum preparation

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Some of the effects of manganese (as the chloride) on the guinea-pig isolated ileum preparation have been examined using an isometric recording system. Manganese (4–64 μM) produced an increase in spontaneous activity, and a slow rise in tension which reached a maximum in 3–4 min; the tension then declined despite the continued presence of manganese. The maximum spasmogenic effect was approximately equivalent to an ED50 dose of bradykinin.

The spasmogenic effect of manganese was reduced by cooling (20° C), tetrodotoxin (10^{-7} g/ml.), *Botulinum* toxin (Type A, 8×10^4 MLD (mouse) in 10 ml.), procaine (10^{-4} g/ml.), pempidine (2×10^{-5} g/ml.), and hyoscine (10^{-7} g/ml.). It was potentiated by mipafox (10^{-5} g/ml.).

After exposure of ileum (pretreated with mipafox, 10^{-5} g/ml.) to manganese (8 μM and 64 μM) for 10 min, the bath fluid was found to possess spasmogenic activity when tested on further pieces of ileum (pretreated with mipafox, 10^{-5} g/ml. and in the presence of tetrodotoxin, 10^{-7} g/ml.). Using ileum so treated and the rat blood pressure (Straughan, 1958), this activity was subjected to parallel biological assay in terms of acetylcholine. The amount of activity released in the presence of manganese was greater than that released spontaneously from the ileum ($P<0·05$). The relative potency of samples and standard was equal on the two preparations.

The log dose : effect lines of the bath fluid samples and acetylcholine standard did not deviate from parallelism in the assays on the ileum ($P>0{\cdot}1$) or in those on the rat blood pressure ($P>0{\cdot}1$). The slopes of the log dose : effect lines in the latter experiments were shallower than those in the former.

The spasmogenic activity of both bath fluid samples and a standard acetylcholine solution on the ileum was abolished after exposure to hyoscine (10^{-8} g/ml.). It was also abolished by boiling in N/6 sodium hydroxide but unaffected by boiling in N/6 hydrochloric acid.

These results indicate that the spasmogen released by manganese is acetylcholine and suggest that the mechanism of this release requires the functional integrity of intramural cholinergic nerves.

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The effects of agroclavine, an ergot alkaloid, on pregnancy and lactation in the rat

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The oral administration of agroclavine has potent effects on early pregnancy and lactation in mice (Mantle, 1968 and personal communication). These observations have been confirmed in the rat and the mechanism of action has been investigated. Purified agroclavine* obtained from fermentation of the ergot *Claviceps fusiformis* (Loveless) was dissolved in 3% tartaric acid solution. The stock solution was added to ground pellet diet to give a concentration of agroclavine 10 mg/100 g diet. Daily food intake ranged from 15 to 25 g and did not differ significantly from that of control animals fed on milled diet containing equivalent amounts of tartaric acid.

Female rats were mated with proven males and the date of mating determined by the presence of sperm in the vaginal smear. Of sixty-one rats mated, twenty-six were placed on agroclavine diet from days 1 to 5 of pregnancy, fifteen were given a similar diet on days 6 to 10 and the remainder served as untreated controls. In the control group eighteen rats (90%) produced normal litters at term, as did eleven (73%) of the rats given agroclavine from days 6 to 10 of pregnancy. None of the rats treated during the first 5 days of pregnancy produced litters and macroscopic examination of the uterus indicated that implantation had probably not occurred in these animals. In contrast, in another group of five rats, agroclavine (2 mg) was administered daily by intraperitoneal injection from days 1 to 5 of pregnancy and all succeeded in bringing litters to term.

A group of twenty-eight healthy lactating rats were placed on the agroclavine diet from days 5, 10 or 15 of lactation. In all cases lactation was greatly reduced within 24 hr and within 48 hr the inhibition was complete. Healthy foster litters given to these mothers were unable to obtain milk during suckling. The administration of prolactin (50 i.u. daily), ACTH (5 i.u. daily) and oxytocin (0·5 i.u. four times daily) was effective in restoring

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lactation to levels between 16 and 68% of control values obtained in untreated animals.

Cannulation of the mammary ducts was performed in a small group of rats under Avertin (bromethol) anaesthesia. Injection of agroclavine (2 mg, I.V.) failed to inhibit the rise in intra-mammary pressure produced by threshold doses of oxytocin. Failure of the milk-ejection response to oxytocin can thus probably be excluded as the cause of the inhibition of lactation observed in agroclavine-treated animals.

Toxic and behavioural effects apparent in animals treated with larger doses of agroclavine were not observed in the animals used in this study. Evidence showing that the effects on pregnancy and lactation may possibly be central in origin and mediated at hypothalamic or pituitary level will be presented.

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Uptake of ^{3}H-gamma-aminobutyric acid (GABA) by rat cerebral cortex

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Recent neurophysiological studies in the cerebral cortex have led to renewed interest in the possibility that GABA may act as an inhibitory transmitter substance in the brain (Krnjević & Schwartz, 1967). Jasper, Khan & Elliott (1965) claimed that the rate of release of GABA from the cat cerebral cortex was related to the state of activation of the brain. Attempts to confirm these results in our laboratory, however, have been largely unsuccessful. Previous studies have established that GABA is accumulated by brain slices (Elliott & van Gelder, 1958; Tsukada, Nagata, Hirano & Matsutani, 1963). The presence of such an uptake process might be responsible for the difficulties encountered in detecting an efflux of GABA from the surface of the cortex. For this reason the characteristics of the GABA uptake process were investigated.

Slices of rat cerebral cortex ($0{\cdot}1 \times 0{\cdot}1 \times$ approx 2 mm) were prepared with a McIlwain tissue chopper, suspended in Krebs phosphate solution and distributed volumetrically. Cortical slices equivalent to 10 mg net weight were incubated with GABA-2,3-^{3}H(5×10^{-8} M) in 10 ml. of oxygenated Krebs phosphate medium. The tissue was then collected by rapid filtration, washed with 5 ml. of ice-cold medium and the total radioactivity was determined by liquid scintillation counting.

There was a rapid accumulation of radioactivity in the tissue, resulting in a tissue/medium ratio of almost 100 : 1 after incubation for 60 min. Ion-exchange and paper chromatographic analyses indicated that more than 95% of the radioactivity accumulated in the tissue was present as unchanged ^{3}H-GABA. The uptake of ^{3}H-GABA exhibited saturation kinetics over a range of external GABA concentrations from 10^{-6} M to 10^{-4} M, with an apparent *Km* for GABA $= 2{\cdot}2 \times 10^{-5}$ M and $V_{max} = 0{\cdot}115$ μmoles/g of cortex per min. When cortical slices were incubated in a high concentration of ^{3}H-GABA (2×10^{-4} M), a net uptake of GABA into the tissue was demonstrated by chemical assay. The net uptake was equivalent to more than twice the normal endogenous GABA content of the tissue, showing that the accumulation of ^{3}H-GABA did not merely represent an exchange between

exogenous and endogenous amino-acid. The uptake of ^{3}H-GABA was temperature dependent, being markedly reduced at 0° C and optimal at 25° C. Replacement of sodium chloride by choline chloride or by sucrose reduced the uptake of ^{3}H-GABA to less than 2% of the control values. A large reduction in uptake also occurred when the tissue was incubated with 2,4-dinitrophenol (10^{-3} M) or with ouabain (10^{-5} M). The uptake of ^{3}H-GABA (5×10^{-8} M) was not affected by the presence of L-glutamate, L-glycine, L-aspartate, or β-aminobutyrate at concentrations of 10^{-3} M. A significant inhibition of uptake was produced by L-alanine and L-histidine (10^{-3} M), and by β-hydroxy-GABA and β-guanidinopropionic acid (10^{-4} M).

The results confirm the existence in brain tissue of a highly efficient and specific uptake mechanism for GABA. The process shows many of the characteristics of an active transport system. It is able to concentrate GABA against a considerable concentration gradient, shows saturation kinetics, is sodium and temperature dependent and can be inhibited by metabolic poisons.

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The pharmacology of cortical repetitive after-discharges

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There is now strong evidence for the existence of two ascending cholinergic pathways in the mammalian brain. One pathway is the non-specific ascending reticulo-cortical (arousal) system and the other is a more restricted repetitive after-discharge (A–D) pathway from specific thalamic nuclei to the appropriate primary receiving areas of the cortex. Little is known about the thalamo-cortical tract, but it may be responsible for altering the local excitability of a cortical receiving area after the arrival of a primary afferent signal (Chang, 1950). The cortical nerve endings associated with this pathway probably release acetylcholine (ACh) and excite deep cholinoceptive pyramidal cells (Collier & Mitchell, 1967; Krnjević & Phillis, 1963).

The present experiments compare the effect of topically and intravenously administered drugs on the evoked A–D responses and on ACh release from the same region of the cortex. Surface and unit A–D responses, evoked by stimulation of a peripheral nerve, have been recorded simultaneously using surface electrodes and extracellular micro-electrodes. The evoked firing of single units corresponded to surface positive A–D waves and both were affected in a similar way by the application of drugs.

The results from fourteen cats, anaesthetized with sodium pentobarbitone, are summarized in Table 1. Except for atropine, which caused an increase in the primary evoked potential, the compounds tested all appeared to have an action only on A–D responses.

TABLE 1

Drug	Change in amplitude of A–D response (×control amplitude)	Change in ACh release from somatosensory cortex (× control release)
Acetylcholine (0·5 mM, topical)	2·5	—
Acetyl-β-methylcholine (5·0 mM, topical)	3·0	—
T.M.A. (10·0 mM, topical)	1·6	—
Eserine (0·1 mM, topical)	3·0	>10
Atropine (6·0 μM, topical)	0·05	5–8
T.E.C. (2·0 mM, topical)	0·3	0·2
Chloralose (3·0 mM, topical)	5·0	—
(13·0 μ-mole/kg, I.V.)	Abolished	Abolished

Noradrenaline and 5-hydroxytryptamine had no effect on A–D responses when applied topically in concentrations up to 3·0 mM. The effects of the compounds listed in Table 1 on A–D responses and on the release of ACh from the same cortical area support the suggestion that transmission at A–D synapses is effected by ACh.

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Effect of chlorpromazine on glucose and nucleotide metabolism in mouse brain

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The mechanism by which chlorpromazine depresses brain function is not understood, but on the basis of *in vitro* experiments Dawkins, Judah & Rees (1959) have suggested that uncoupling of oxidative phosphorylation induced by the drug could produce such an effect.

Uniformly ^{14}C labelled glucose was given to mice intraperitoneally (0·2 mc; 12·0 mg/kg) 1 hr after either chlorpromazine (10 mg/kg) or saline were injected intraperitoneally. In those animals pretreated with chlorpromazine there was diminished incorporation of isotope into brain proteins and lipids, with a relative increase in isotope in the remaining acid soluble fraction ($n=12$; $P=0{\cdot}001$–$0{\cdot}01$). This is interpreted as a decrease in protein and lipid synthesis. Analysis of the acid soluble fraction by non-isotopic methods revealed increased amounts of glucose and glucose phosphate in the tissues of mice given chlorpromazine.

Chlorpromazine uncouples oxidative phosphorylation *in vitro*. Thus there is a consequent decrease in the formation of high energy phosphate. A failure of energy production could explain the inhibition of such energy dependent syntheses as those of protein and lipid. Direct measurements of ADP, AMP and ATP, however, showed normal levels and ATPase activity was not inhibited in the tissues of animals treated with chlorpromazine *in vivo*. Thus mechanisms other than alterations in availability of high energy phosphate must be involved in the metabolic changes observed.

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DEMONSTRATIONS

Some observations on the use of aspirin in certain thrombotic diseases

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Vascular occlusive disease occurring simultaneously in the lungs and hearts of rabbits resulted from the production of intravascular platelet clumping. Platelet clumping was produced by three dissimilar experimental methods, but the lesions formed were identical in each case. In the hypersensitivity reactions that occur in rabbits that receive a single intravenous injection of horse serum cortisone prevented platelet clumping and the lesions (Hughes & Tonks, 1956, 1959, 1962).

In certain types of human pulmonary infarction similar lesions are found in the lungs and myocardium (Hughes & Tonks, 1968). These patients respond dramatically to corticotrophin, adrenocorticosteroids or aspirin. More than 1,000 such patients received long-term maintenance treatment with aspirin in the form of delayed release preparations (most frequently Caprin, West Silten Pharmaceuticals Ltd.). The dose was from 4 to 6 g daily.

Oral aspirin delays platelet clumping in human blood and increases the blood ATP : ADP ratio proportionately (Davies, Hughes & Tonks, 1968). Hence this action may contribute to its clinical effect in a disease believed to result from intra-vascular platelet clumping.

Gastro-intestinal bleeding has been considered to be an important hazard of aspirin therapy. In addition, a few cases of marrow dyscrasias have been reported (Nieweg, Bouma, de Vries & Jansz, 1963; Pretty, Gosselin, Colpron & Long, 1965; Wijnja, Snijder & Nieweg, 1966) and "analgesic" nephritis has been suspected (Prescott, 1965, 1966). Fifty-nine patients taking a mean daily dose of 4·98 g of aspirin have been reviewed regularly for up to 6 years. Gastro-intestinal bleeding occurred in three cases, one of whom had hiatus hernia and another had previously suffered from duodenal ulceration.

No case of bone marrow dysfunction was encountered, nor did pyelonephritis, azotaemia or renal hypertension occur.

With a daily dose of 5·4 g aspirin in the form of Caprin plasma salicylate levels of about 25 mg/100 ml. are achieved in 4 to 5 days. Measurement of urinary loss of salicylate is a convenient method of assessing the amount absorbed from the delayed release preparation.

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Ionic transport by frog skin: experience of a class experiment

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Frog skin demonstrates many of the features of electrogenic sodium transport. Its *in vitro* study has proved a feasible student exercise using 3 units as part of a rota system in a fairly large practical class, using an inexpensive voltmeter and current source and an Ussing type chamber (Barry, 1967; Barry & Davies, 1967). Among other minor modifications, in this apparatus the frog skin is mounted on a spiked ring, which considerably reduces damage to the membrane from rough handling.

Because a class experiment has to be completed within 3 hr, an experimental procedure which would give obvious and rapid responses was needed. The protocol used has been: the skin in the chamber (5 cm^2) was immersed in standard Ringer solution to record control observations for 20 min; mucosal sodium was then, with washing, replaced by choline for 20 min; the skin was again bathed, with washing, in standard Ringer for 15 min; then vasopressin (Pitressin) was added to the serosal side to give 0·4 u./ml. and readings taken for 30 min; 2,4-dinitrophenol was then added to both sides to give 10^{-3} M and changes were observed for another 15 min.

Of fifty-four student experiments performed during the autumn using abdominal skin from predominantly *Rana temporaria* (and a few *Rana pipiens* which gave similar results), forty-two gave adequate readings. Of the remaining twelve, three failed because of a

low initial potential and poor response of the frog skin and nine failed because of errors in following the protocol or in handling the apparatus.

In the forty-two successful experiments initial membrane potential (E) ranged from 6 to 100 mV and short-circuit current (I) from 20 to 310 μA. Subsequent values are expressed as a percentage of the immediately preceding "control" reading. Five and ten minutes after replacing sodium by choline E had fallen to 32 and 30% of its initial value; I was often below 10 μA and so difficult to measure. Ten, twenty and thirty minutes after adding vasopressin, E had risen to 111, 121 and 129%, and I to 113, 126 and 139% of the preceding control reading. Ten minutes after adding dinitrophenol, in terms of the 30 min vasopressin reading, E had fallen to 63% and I to 41%.

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Changes in blood urea levels in beagles fed on various diets

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In man, although individuals on a high-protein diet may show levels of blood urea significantly greater than those eating normal amounts of protein (that is, 1–1·5 g/kg/day), it is generally accepted, possibly without adequate proof, that the blood urea concentration does not rise significantly after a meal of normal size and content (McKay & McKay, 1927; Addis, Barrett, Poo & Yuen, 1947; Cantarow & Trumper, 1962).

Dogs are, however, usually fed on a diet which supplies considerably more protein (per kg body weight) than normal human diet, and they often receive most or all of their daily intake of protein in one meal. Although one authority has recommended that blood samples for urea estimation be taken from dogs after fasting (Bloom, 1960), many workers consider that the influence of diet on blood urea levels in the dog is similar to that in humans—that is, that urea levels show no or only a slight rise after a meal, although they may be persistently raised in animals on a steady high-protein diet (Robin, 1948; McKelvie, Powers & McKim, 1966; Coles, 1967). Hoe & O'Shea (1965) reported that even on a sustained high-protein diet, blood urea values in puppies fell to normal levels after 14 days.

In the course of investigations carried out in our own beagle colony and that of another establishment, we were surprised to find that in these animals the blood urea was consistently elevated, often to levels generally considered indicative of pathological abnormality, for many hours after each meal (Street, Chesterman, Smith & Quinton, to be published). The diets used in these investigations were of several types, all routinely used in large dog colonies. Our conclusions were that, to obtain consistent blood urea values in beagles used, for example, in long-term toxicity trials, the animals must be fasted for 10–18 hours (depending on the size and nature of their last meal) before a blood sample is taken.

The post-prandial rise in blood urea in the beagle seems to be not necessarily in proportion to the total amount of protein ingested, but varies somewhat from one diet to another. These differences presumably reflect the source of protein in the diet, the ease with which it is absorbed from the alimentary tract, and possibly the suitability of its constituent amino-acids for normal anabolic processes or for degradation to urea in the liver.

A number of proprietary dog-foods have been fed to beagles in a cross-over study, and the consequent increases in blood urea compared; certain conclusions are drawn regarding their relative merits as sources of nutritive protein.

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Distribution of an anti-acetylcholine drug, phenglutarimide hydrochloride, in the rat and mouse

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Phenglutarimide hydrochloride is used clinically in the treatment of Parkinson's disease, but the site of its action is unknown. Although the drug is helpful in relieving excessive salivation and muscular rigidity, it has no obvious effect on tremor and oculogyric crisis.

The distribution of phenglutarimide hydrochloride labelled with ^{14}C in the 2 and 4 positions on the glutarimide ring has been determined in female albino rats (100–150 g). After the oral administration of the drug (19 μc/mg in a dose of 2·15 mg/kg) 90–100% of the radioactivity is distributed between the gastrointestinal tract and the urine at any time up to 12 hr from the time of dosing. Levels of radioactivity in the tissues are low because the rate of excretion rapidly balances the rate of absorption. Radioactivity is highest in the liver and lowest in the plasma. No labelled drug is detectable in the brain, suggesting that phenglutarimide does not traverse the blood–brain barrier. However, the drug readily crosses the placenta in pregnant animals. A similar pattern of distribution of phenglutarimide is seen in the female albino mouse using an autoradiographic technique. Because oxotremorine-induced tremor in the mouse is not antagonized by phenglutarimide, it is concluded that this glutarimide exerts no central action after oral administration.

Phenglutarimide is not metabolized and 100% is excreted in the urine of rats and mice in 24 hours.