Table 2. CHANGE IN BLOOD GLUCOSE IN ADRENALECTOMIZED CATS AFTER INTRAVENOUS ADRENALINE (5 μg) AND HYDROCORTISONE (1 OR 10 mg)

Exp.	Initial blood glucose mg/100 ml.	Change in blood glucose (mg/100 ml.) at successive time intervals (min) after injection 5	15	20	30	60
		1 mg hydrocortisone				
1	42	10	10	1	12	−2
2	57	5	11	9	6	7
3	42	4	5	3	14	11
4	67	6	−4	7	−3	6
Mean ± S.E.M.	52 (6)	6·3 (1·3)	5·5 (3·4)	5·0 (1·8)	7·3 (3·8)	5·5 (2·7)
		10 mg hydrocortisone				
1	49	15	11	21	17	7
2	52	15	8	8	12	7
3	66	18	8	9	6	13
4	64	26	7	9	15	10
5	55	13	10	13	12	—
Mean ± S.E.M.	57 (3)	17·4 (2·3)	8·8 (0·7)	12·0 (2·4)	12·4 (1·9)	9·3 (1·4)
P value for comparison between no hydrocortisone and 10 mg		0·01–0·001	> 0·10	0·02	0·01–0·001	—

(Table 2), and addition of the increases after 15, 20 and 30 min showed 10 mg to give results significantly different from those when steroid was not injected ($P = 0·01$–$0·001$). There was no significant difference between the mean initial values for the blood glucose in any of the groups. 25 mg hydrocortisone alone was not followed by any increase in blood glucose within 30 min of injection.

Similar but less clear-cut results were obtained in normal cats[13]. The situation in them was perhaps partially obscured by endogenous secretion of adrenaline and hydrocortisone. The rise in blood glucose 5 min after injection appears to depend more on whether intact or adrenalectomized cats are used (being greater in the normal cats) than on the amount of any accompanying steroid[13].

These results show that hydrocortisone can increase the blood glucose concentration (in the setting of its elevation by 5 μg adrenaline) within 15–30 min. The dose of 25 mg steroid to a 2·5-kg cat is comparable (on a basis of body-wt) to 2·0 mg to a 200-g rat, a value equal to or less than that used in many of the classical experiments on gluco-corticoid action. 10 mg hydrocortisone given to a 2·5-kg cat would give a concentration of approximately 2×10^{-5} M if distributed throughout the body water.

The rapidity of this action on blood glucose makes likely a direct action of hydrocortisone on carbohydrate metabolism, rather than that its primary action is elsewhere (say, on nucleic acids). However, it has recently been found[14] that hydrocortisone elevates the concentration of non-esterified fatty acids in venous blood 10 min after the start of a continuous intra-arterial infusion into the human forearm, so it is possible that the effect on glucose is secondary to one on fat metabolism. The present results throw no light on how the effect on glucose occurs. It could be by a recognized action of hydrocortisone, such as increase in gluconeogenesis, or by a disputed effect, such as inhibition of the peripheral utilization of glucose; it could be by some synergism with adrenaline's action on carbohydrate metabolism in the body at large; or it could be by potentiation of the intracranial action of adrenaline that leads to increased blood glucose. However, this last possibility seems unlikely since Sproul[15] found in cats that the central nervous system was unlikely to contribute much to the increase in blood glucose after as large a dose as 50 μg adrenaline intravenously.

Whatever the mechanism of hydrocortisone's action, the present experiments provide an example of the rapid potentiation of an effect of a hormone from the adrenal medulla by one from the adrenal cortex. While the enhancement by steroids of noradrenaline's action on the blood pressure is probably secondary to changes in sodium metabolism[16], the present experiments do provide an example of rapid interaction of these two types of hormone.

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ELECTROMYOGRAPHIC ANALYSIS OF LARYNGEAL ARTICULAR REFLEXES

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IN previous communications[1–3], we have shown that the capsules of all laryngeal joints are directly innervated by articular branches of the laryngeal nerves, the fibres of which terminate in corpuscular and non-corpuscular receptor endings in the articular tissues[2]. We have further shown[3,4] that graded electrical stimulation of the afferent fibres in the articular nerves produces reflex contractions of the intrinsic muscles of the larynx that are associated with movements of the vocal folds. Analysis of the results of these experiments[3,4] in the light of our neurohistological investigations[2] suggested that the larger (low-threshold) articular nerve fibres are mechanoreceptor afferents and that the small (high-threshold) fibres are pain afferents, related respectively to the corpuscular and non-corpuscular articular nerve endings.

Our most recent communication to *Nature*[3] indicated that an electromyographic analysis of laryngeal articular reflexes would be undertaken to obtain more precise data on their mode of operation. We now wish to report on such an investigation, as it confirms and extends our previous observations. The first part of this investigation involves repetition of the electrical stimulation experiments[3] during electromyography: the second part provides electromyographic evidence of mechanoreceptor reflexes provoked by laryngeal joint movement.

All experiments were conducted on intact cats kept lightly anæsthetized with pentobarbitone. Neither tracheotomy nor intubation was performed: instead, the animals were allowed to breathe an oxygen-enriched air mixture spontaneously through an intrapharyngeal tube. The nerves, and all other exposed tissues, were kept submerged in warm mineral oil. The animals were kept warm on a thermostatically regulated, heated operating table. Operative procedures were performed with microsurgical instruments and a Zeiss operating microscope. All responses were tested at various phases of the spontaneous respiratory cycle.

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Electrical stimulation of articular nerves. In 8 cats, the articular branches of the left internal and recurrent laryngeal nerves (and of the ramus communicans) innervating the thyrohyoid, thyro-epiglottic, crico-arytenoid and cricothyroid joints were directly stimulated electrically with single rectangular pulses of graduated amplitude and duration, through a bipolar electrode mounted in a stimulus isolation probe. After removing the left thyroid lamina, synchronous bipolar electromyograms were recorded (with stainless-steel needle electrodes, insulated except at their tips) from extrinsic (sternohyoid, geniohyoid, cricothyroid) and intrinsic (lateral thyro-arytenoid, lateral crico-arytenoid, posterior crico-arytenoid) laryngeal muscles on a Grass polygraph with appropriately adjusted frequency-response characteristics. Simultaneous recordings were made of the animal's respiratory movements in thorax and abdomen, and of its electrocardiogram. The effects of articular nerve stimulation were compared with those of excitation (with comparable stimulus parameters) of mucosal branches of the laryngeal nerves, of muscular branches of the recurrent laryngeal nerve, of the external laryngeal nerve, and of the trunks of the superior and recurrent laryngeal nerves (and the ramus communicans) at various sites. The effects on the muscular responses to articular nerve stimulation of section of the nerves distal and proximal to the stimulating electrodes were also examined, as were the effects of changing general anæsthesia.

Low-intensity stimulation of each articular nerve resulted in a brief (less than 0·5 sec) burst of motor unit potentials in some of the laryngeal muscles (most frequently the adductors) that was superimposed on their spontaneous activity, irrespective of the phase of the respiratory cycle at which stimulation was performed. The occurrence of such bursts in some muscles was associated with an equally brief reduction in spontaneous motor unit activity in others. The usual response from the nerves to the cricothyroid joint was a burst of motor unit potentials occurring simultaneously in the lateral thyro-arytenoid, latera crico-arytenoid and cricothyroid muscles (and sometimes, but at lesser amplitude, in the sternohyoid muscle as well), with coincident reduction of posterior crico-arytenoid activity—that is, transient adductor facilitation with reciprocal abductor inhibition. Similar responses (but not involving the strap muscles) were generally obtained also from the nerves to the crico-arytenoid joint; but sometimes abductor responses (involving motor unit bursts in the posterior crico-arytenoid muscle with coincident inhibition of the other intrinsic muscles) were elicited with the same stimulus parameters. Low-intensity stimulation of the nerves to the thyrohyoid and thyro-epiglottic joints always led to adductor responses, accompanied by synchronous bursts of activity in both the sternohyoid and geniohyoid muscles.

High-intensity stimulation of any of the articular nerves (or of the mucosal branches of the laryngeal nerves) always produced more diffuse and prolonged reflex effects. These involved an increase in muscle tone generally, intense motor unit activity in the adductor and strap muscles associated with laryngeal spasm and interspersed with repeated higher voltage bursts due to rapid swallowing movements, apnœa followed by hyperpnœa, tachycardia, and dilation of the pupils.

The threshold for the production of the laryngeal muscular responses to low-intensity articular nerve stimulation was higher than that of the motor nerves to the same muscles, but considerably lower than that of the mucosal branches of the laryngeal nerves. All responses were unaltered by section of the articular nerves distal to the point of stimulation, but were uniformly abolished by proximal nerve section. It was also noted that the responses to low-intensity stimulation could only be obtained with light general anæsthesia, and that with increasing anæsthesia these responses were abolished before those provoked by high-intensity stimulation of the same nerves. The reverse sequence of events was obtained with diminishing general anæsthesia.

Passive movement stimulation of articular mechanoreceptors. In eleven cats, the left cricothyroid joint was isolated from the lamina of the thyroid cartilage and from the attachments of the cricothyroid and inferior constrictor muscles. The thyroid lamina was then removed on the left, the intra- and extra-laryngeal branches of the superior laryngeal nerve being carefully preserved. Non-elastic threads were tied through the residual articulated fragment of the inferior cornu of the thyroid cartilage, and graduated passive movements of the joint were performed by attaching weights (ranging from 5 g to 20 g) to these threads, over pulley surfaces. Movements were performed in four planes mutually at right angles (that is, in cranial, caudal, anteromedial and anterolateral directions). Electromyograms, and recordings of respiratory movements and of the electrocardiogram, were made as in the electrical stimulation experiments (*vid. sup.*). The effects on the responses to passive movement of infiltration of the joint capsule with 2 per cent 'Lignocaine' hydrochloride solution, of electrocoagulation of the joint capsule, of changing general anæsthesia, of section of the nerves to the cricothyroid joint, and of ipsilateral and bilateral section of the internal, external and recurrent laryngeal nerves were also examined.

Passive movements of the cricothyroid joint—but in caudal and anteromedial directions only—led to brief bursts of motor unit potentials at the onset of movement in the lateral thyro-arytenoid, lateral crico-arytenoid and cricothyroid muscles with coincident inhibition of spontaneous activity in the posterior crico-arytenoid muscle. Similar bursts of activity occurred at lower voltage in the sternohyoid muscle, usually with coincident inhibition of geniohyoid activity—especially with caudal displacement of the joint. Release of the displaced joint led to an identical response pattern at the moment of release. No such effects resulted from movements of the joint in cranial and anterolateral directions. The responses to passive movement of the cricothyroid joint thus resemble —in their short time course and distribution of the laryngeal musculature—the effects of brief single-pulse stimulation of the low-threshold fibres in the articular nerves innervating the joint.

The responses to passive movement of the joint were reversibly abolished (for a period of 40–60 min) by infiltration of the joint capsule with a local anæsthetic (2 per cent 'Lignocaine' hydrochloride) solution. They were irreversibly abolished by electrocoagulation of the joint capsule, or by section of the articular nerves. Bilateral section of the internal laryngeal nerves had no effect on the muscular responses. Bilateral section of the external laryngeal nerves abolished the responses in the cricothyroid muscle only. Ipsilateral section of the recurrent laryngeal nerve below the larynx abolished the responses in all the muscles—including the cricothyroid and strap muscles. The responses could only be elicited at light stages of general anæsthesia, and were reversibly abolished by increasing anæsthesia—as was the case with the responses to low-intensity stimulation of the articular nerves.

The findings in these experiments confirm that the articular nerves innervating the laryngeal joints contain low- and high-threshold afferent fibres[3,4], discharges in which provoke reflexly co-ordinated changes in the tone of the laryngeal muscles[3,4]. They further indicate that the low-threshold (larger diameter) articular afferents, at least in the case of the cricothyroid joint, are driven by rapidly adapting mechanoreceptor endings embedded in the capsular tissues[2,3] which are selectively stimulated by movements of the joint in specific directions; whereas the high-threshold (small diameter) afferents provide a pain-conducting system from non-corpuscular nerve endings[2,3] in the joint capsule They also confirm that most of the afferents from the cricothyroid joint enter the ipsilateral recurrent laryngeal nerve, as we have indicated else-

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where[1,2], rather than the internal or external laryngeal nerves.

These investigations now provide clear evidence that articular mechanoreceptor reflexes are involved in the normal co-ordination of activity in the laryngeal muscles, as we have suggested previously[1,3]. They also indicate that should abnormal situations arise in the larynx such that the pain-receptor system in the joint capsules (as well as, or apart from, that in the mucosa) be stimulated, then distorted reflex patterns of laryngeal muscular activity are provoked. Indeed, the stage has now been reached when laryngologists, phonologists and respiratory physiologists might well consider the possible role of laryngeal articular reflexes in the mechanisms of speech and respiration.

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KININASE INHIBITION BY A FLUORESCENT SUBSTANCE PREPARED FROM LIVER

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IT has been reported that a non-coagulable and fluorescent protein (polypeptide) fraction prepared from ox liver has several interesting biological properties. This fraction, which has been given the name 'urgocyton'[1], interferes with the growth of vaccinia virus *in vivo* and it gives protection in mice against infections with *Escherichia coli*[2,3]. Furthermore, it inhibits mitotic activity of human cells grown *in vitro*[4]. It also gives mice certain protection against cold stress[5]. A similar effect has also been reported for material prepared from ox blood[6]. As there is no explanation for these effects, it seemed desirable to search for significant actions of urgocyton in simple, controllable biological systems. We have thus investigated its possible relationship to the formation and inactivation of bradykinin.

In the discussions on the action of the plasma kinins and their possible biological significance, interest has been focused on the mechanisms for their formation and destruction[7]. An analysis of the conditions influencing or governing formation and destruction of these very active substances is probably essential for an evaluation of their physiological and pathological significance. Plasma kinin-forming and plasma kinin-destroying enzymatic activities have been identified in several biological fluids and tissues[7–11]. The great plasma kinin inactivating capacity of blood, of other biological fluids and of several tissue homogenates[10,11] is a striking and possibly important characteristic of such materials.

The investigations carried out with urgocyton revealed that this material caused a marked inhibition of the bradykinin-destroying activity of hæmolysed red blood cells and also, to some extent, of the bradykinin-destroying activity of plasma.

A ninhydrin-negative and fluorescent substance prepared by acid hydrolysis of urgocyton was shown to have kininase-inhibiting properties similar to those of urgocyton itself.

Methods for preparing urgocyton on a small scale. The method is a modification of one previously described[4]. One part of freshly minced ox liver was heated with 2 parts of distilled water at 90°–100° C for 30 min. When cool, the mixture was filtered and the residue washed with 1 part of water. The combined filtrates were freeze-dried and 2 parts of 70 per cent aqueous ethanol was afterwards added. The mixture was left at room temperature and filtered the next day. The *p*H of the filtrate (measured after a 4-fold dilution with water) was adjusted to 8·5 by addition of 2 N ammonia. Barium acetate dihydrate (1/200 part), dissolved in a small amount of water, was added and then ethanol up to a final concentration of 92 per cent. After storage at room temperature, the precipitate was removed by centrifugation the following day. The precipitate was dissolved in 0·1 part of water and the mixture passed through a suitable column of 'Amberlite *IR* 120 (NH_4)' to remove barium ions. The effluent was dialysed against water at +2° C for 48 h. On freeze-drying a white solid was obtained, the yield of which varied somewhat from batch to batch, but was usually about 0·05 per cent of the weight of the liver. The fluorescence of the material was measured in a Farrand fluoro-spectrophotometer. In 0·01 M phosphate buffer *p*H 7·0, it exhibited a fluorescence maximum at 440 mμ and an excitation maximum at 340–350 mμ.

Isolation of a fluorescent substance from urgocyton. 10 g of urgocyton was hydrolysed in a sealed tube at 110° C for 24 h with 6 N hydrochloric acid. The hydrolysate was concentrated to dryness in a vacuum under nitrogen and the residue dissolved in 200 ml. water (*p*H between 1–2). The solution was treated with 20 g of charcoal ('Norit') for a few hours and the charcoal filtered off and washed with water until free of chloride ions. The fluorescent substance was then eluted from the charcoal with 90 per cent aqueous phenol. The fluorescent eluant was added ether and water to displace the fluorescent material into water. After concentration of the aqueous phase in vacuum, the material was subjected to paper chromatography on Whatman 3 *MM* in butan–1–ol–ethanol (96 per cent)–aqueous ammonia (sp.gr. 0·91)–water (4 : 1 : 2 : 1, by volume). The strongly bluish fluorescent band was eluted with chloroform–methanol–aqueous ammonia (17 per cent w/v) (2 : 2 : 1, by volume) and the concentrated solution rechromatographed. The fluorescent band gave a negative ninhydrin reaction. The eluate of the band was concentrated to dryness, redissolved in water and treated with 0·6 g charcoal. The charcoal was washed with water until free of chloride ions and the fluorescent material again eluted with ethanol–water–aqueous ammonia (sp.gr. 0·91) (50 : 48 : 2, by volume). On concentration to dryness 5·5 mg of a nearly colourless substance was obtained. On Whatman No. 1 paper in butan-1-ol-ethanol–aqueous ammonia (sp.gr. 0·91)–water (4 : 1 : 2 : 1, by volume) and containing 0·2 per cent ammonium chloride, it had R_F 0·25. In thin-layer chromatography using cellulose '*MN* 300 *G*' (Macherey, Nagel and Co., Düren, Germany) as supporting medium and the above solvent system, it had an R_F of 0·49. In paper electrophoresis in a borate–potassium chloride buffer, *p*H 8·6 (500 ml. 0·2 M potassium chloride + 500 ml. 0·2 M orthoboric acid + 236 ml. 0·1 M sodium hydroxide in a total volume of 2,000 ml.), it moved 4·5 cm in 16 h towards the anode at 1·7 V/cm. In 0·01 M phosphate buffer it exhibited a

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