A simplified method for preparing a pithed cat

SIR,—Most investigators who use the spinal cat follow Dale's method as described by Burn (1952), which involves section of the highly vascularised dorsal spinal muscles, which cling tenaciously to the vertebrae, and resection of the vertebral bone to expose the spinal cord. Recently, Zarro & DiPalma (1964) described another method for preparation of the spinal cat for use in studies on the superior cervical ganglion or the nictitating membrane. The spinal cord was transected through the anterior atlanto-occipital aperture which is readily accessible after the surgical procedures in the neck necessary to expose the superior cervical ganglion. However, since the surgical procedures are extensive, the method is not convenient when a study is to be made involving organs or tissues not located in the neck.

In addition to the disadvantages listed above, it was discovered that when spinal cats in which the spinal cord had not been pithed were used for prolonged experiments (up to 8 hr), large cyclic changes in blood pressure sometimes occurred (4 out of 13 cats). These changes appeared and disappeared at random intervals, the mean blood pressure varying as much as 100 mm Hg. When they occurred, they interfered with the orderly course of the experiment. The cyclic blood pressure changes could be eliminated by an injection of procaine into the spinal cord, which suggests that they were due to activity of spinal vasomotor centres. The following procedure eliminates some of these disadvantages.

The cat is anaesthetized with diethyl ether. After tracheotomy the cat is maintained under light ether anaesthesia by appropriate adjustment of a valve on an etherising bottle which is connected to the tracheal cannula. The head is flexed forward maximally and a midline incision of the skin of the nape of the neck is made. The superficial platysma muscles directly caudad to the lambdoidal ridge of the cranium are doubly ligated and severed and the underlying occipital muscles are scraped from the occipital bone with the blunt end of a scalpel holder, exposing the posterior atlanto-occipital membrane. The membrane is slit and pushed aside to expose the spinal cord. Ether administration is discontinued, and artificial respiration is started. A metal hook is passed around the spinal cord and quickly pulled upward to sever the cord. The brain is destroyed by passing a blunt probe through the foramen magnum. The spinal cord is destroyed by passing a flexible probe into the spinal column. A suitable probe can be cut from a spiral wire spring 4·0 mm in diameter, of the kind commonly sold to clean obstructed sink drains. A cotton wad saturated with petroleum jelly is packed into the spinal column, and the atlanto-occipital opening is corked. After placing a pledget over the wound, the cut edges of the skin are stitched together.

The method described here for pithing the spinal cord proved to be notably satisfactory with 116 cats. Cyclic changes were observed in only 3 of these cats, possibly because there was incomplete destruction of the spinal cord. The mean blood pressure of this group was 69 $\pm$ 14 (s.d.) mm Hg. This method has several advantages over those commonly used. It requires a minimum of surgery, and hence the cat is subjected to less trauma and a shorter period of hazardous ether anaesthesia. In experienced hands, the entire procedure can be completed within 15 min. The method has a further advantage over the anterior approach, even when the preparation is to be used for studies on the superior cervical ganglion, since the spinal cord can be pithed more readily through the posterior atlanto-occipital opening than the anterior.

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Concerning the regulation of some diverse biochemical reactions, underlying the inflammatory response, by salicylic acid, phenylbutazone and other acidic antirheumatic drugs

SIR,—Recent *in vitro* studies of the properties of non-steroid anti-inflammatory: antirheumatic drugs have indicated that, in addition to their analgesic properties, they might inhibit several chemical reactions *in vivo* which probably participate in the overall development of *signa inflammationis*, the subsequent formation of granulation (scar) tissue and wound repair (Spector, 1964; Garattini & Dukes, 1965; Whitehouse, 1965). The biochemical reactions include (i) the mitochondrial biosynthesis of adenosine-5′-triphosphate(ATP) ("oxidative phosphorylation"); (ii) the formation of histamine by substrate-specific histidine decarboxylase(s); (iii) the hydrolysis of proteins or amino-acid esters, or both, by enzymes resembling trypsin (EC no. 3.4.4.4) in their substrate-specificity, for example, the Hageman factor (Schoenmakers, Matze, Haaner & Zilliken, 1964), the kinin-forming enzymes (Webster & Pierce, 1961), and thrombin and plasmin: fibrinolysin (Scheraga, Ehrenpreis & Sullivan, 1958).

These three particular processes, though superficially unrelated in their chemistry, appear to have at least two features in common: firstly their sensitivity to mM concentrations of salicylic acid and certain other acidic anti-inflammatory drugs such as phenylbutazone, cinchophen, indomethacin and flufenamic acid; and secondly, the involvement of an ϵ-amino-group (belonging to a lysine residue in the enzyme protein or protein substrate) in the enzyme-substrate interaction. Where the relation between chemical structure and the ability to inhibit these enzyme reactions has been investigated (see Whitehouse, 1965), it is notable that non-acidic derivatives of these drugs, such as salicylamide, *N*-arylanthranilamides and the amide of indomethacin, are unable to substitute for the parent acid as effective anti-inflammatory drugs or inhibitors of these enzyme systems (although they may still carry analgesic activity). We therefore believe that some of the apparently diverse effects of salts of salicylic acid and other anti-inflammatory acids upon enzyme systems, especially those implicated in the inflammatory response of animal tissues, are due to "neutralisation" of essential lysyl ϵ-amino-groups by the anionic form of these drugs. The evidence for this hypothesis is summarised, as follows.

A. Drugs known to selectively inhibit mitochondrial ATP biosynthesis without affecting mitochondrial respiration (so-called "uncoupling agents") are either (i) acids able to partition from an aqueous phase into the mitochondrial lipid phase, for example, 2,4-dinitrophenol and the acidic anti-inflammatory drugs considered here, or (ii) compounds able to interact with an amino-group adjacent to a thiol group, for example, certain trivalent arsenicals, and carbonyl-cyanide-phenylhydrazones (Heytler, 1963; Whitehouse, 1965). We discovered that several other compounds able to react with free amino-groups under mild conditions (quasi-physiological pH, room temperature) in an aqueous medium,