Recent work in this laboratory indicates that within the epidermis proper the greatest arginase activity is localized in the cells of the upper portions of the Malpighian layer and seems to reach its maximum in the granular layer.

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A Method of Intravenous Injection of Drugs from a Distance in Conditional Reflex Studies

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The study of higher nervous activity through the medium of conditional reflexes usually requires that the animal be isolated from both the experimenter and all irrelevant stimuli. Procedures requiring a series of injections of measured amounts of a drug have not been possible because of the lack of a satisfactory technique. This deficiency had to be overcome before we could investigate cardiac, respiratory, and salivary conditional reflexes in which acetylcholine is used as the unconditional stimulus. In the studies planned, repeated intravenous injections of the drug are necessary, but it is essential to avoid any disturbance of the animal during the course of the injections. The technique described below permits the intravenous injection of measured quantities of a drug while the investigator is separated from the animal, thus avoiding the disturbing effect of the investigator's presence, repeated introduction of the needle, etc.

The authors (*1*) recently described a method for injecting acetylcholine into the superior sagittal cerebral venous sinus, in the isolated, unanesthetized dog. This technique has been modified by the use of plastic tubing inserted into the external jugular or radial vein, as described here. Fitzpatrick, Schnabel, and Peterson (*2*) used plastic tubing for cardiac catheterization, by introducing the tubing into the heart through the external jugular vein. In our experiments a very fine-bore plastic tube about 3 in. long with a steel wire stylet already in place is inserted into the external jugular vein of an anesthetized dog. This is accomplished by first introducing a special 16-gauge needle into the vein and then passing the plastic tubing through the needle. The needle is pulled out over the

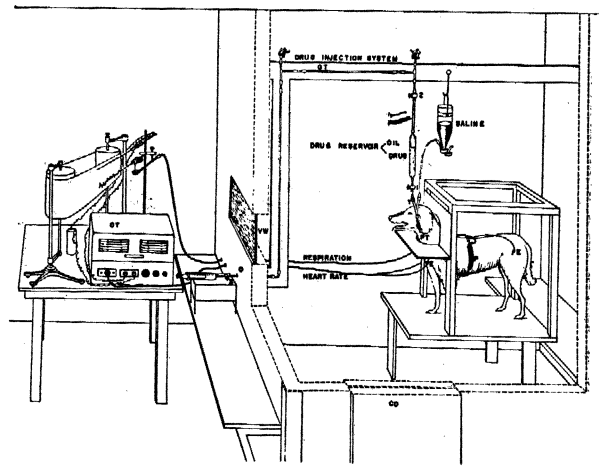

FIG. 1. Arrangement of injection apparatus and dog during experiment: *E*, electrodes for heart rate; *P*, pneumograph; *PT*, plastic (polyethylene) tubing; *T*, Y-tube; *GT*, oil-filled glass tubing; *CT*, cardiotachometer; *CD*, camera door; *O*, outlet for oil-filled tubing; *VW*, viewing window—one-way vision from outside camera; 1, 2, and 3, stopcocks.

tubing, which may be left in the vein for several days. The protruding end of the stylet is then bent at its point of exit so as to prevent accidental movement inward, lest it extend beyond the inner end of the tubing and injure the vein. The bent end of the stylet is sutured to the skin. On the following day, after the dog has recovered completely from the anesthesia, it is placed in the camera under slight restraint and with the injection apparatus applied as illustrated in Fig. 1. A 23-gauge needle with shortened bevel is inserted into the plastic tube (Fig. 1, *PT*) after removal of the stylet, and the needle is fixed to the neck by means of adhesive tape. A metal Y-tube (Fig. 1, *T*), with adapters at the end of the stem and on the arms, is attached to the needle. One arm is connected to a saline solution, and the other to a drug reservoir, which is a glass tube 6 in. long and ½ in. in diameter, tapered at both ends to permit the attachment of

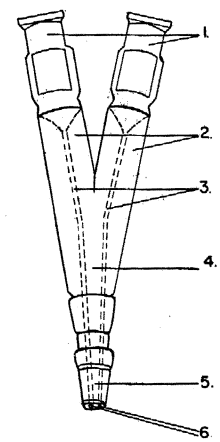

FIG. 2. Y-tube showing details of construction: 1, adapters of arms; 2, arms of Y-tube; 3, inner tubes; 4, stem of Y-tube; 5, adapter; and 6, open ends of inner tubes.

rubber tubing. The Y-tube serves merely as a sheath to contain 2 smaller tubes completely isolated from each other, extending from the arms of the Y and terminating at the end of the stem (Fig. 2). A constant flow of saline is thus maintained through one arm of the Y and the plastic tubing, to prevent occlusion of the latter by blood clotting. At the lower end of the drug reservoir there is a glass stopcock (Fig. 1, 1) which, when closed, prevents the escape of the drug solution until ready for use. The upper end of the drug reservoir is joined by means of plastic tubing to glass tubing (Fig. 1, *GT*) filled with mineral oil. Oil is also added at the upper end of the drug reservoir, so as to float on the surface of the drug solution (Fig. 1). A glass stopcock (Fig. 1, 2) at the end of the glass tube permits detachment of the reservoir for cleaning purposes and for the replenishment of the drug, without loss of oil. The distal end of the oil-filled glass tube passes through an outlet in the wall of the camera (Fig. 1, *O*) in which the dog is isolated, and the escape of oil is prevented here by means of another glass stopcock (Fig. 1, 3). During

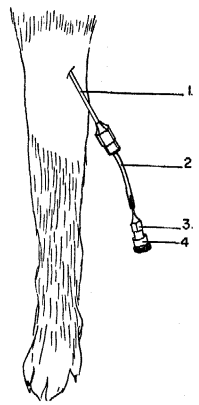

FIG. 3. Method of inserting plastic tubing into radial vein of dog: 1, special 16-gauge needle in vein; 2, polyethylene tubing filled with saline and threaded into vein through large needle; 3, 23-gauge needle; and 4, Luer lock cap.

injection, with the drug reservoir in place, and stopcocks 1 and 2 open, a syringe containing mineral oil is attached to the adapter-equipped distal end of the oil-filled tubing. Then stopcock 3 is opened. The injection of a measured amount of oil displaces an equal amount of drug from the reservoir into the external jugular vein.

This method of injection has also been carried out in the radial vein. No anesthesia is required here, as this vein is readily accessible in the foreleg and easily pierced by the special 16-gauge needle, with little resistance on the part of the dog. The leg used is restrained to limit its range of movement to several inches. The plastic tubing to be inserted into the vein is prepared by insertion of a ½-in., 23-gauge needle into one end, and filling the tubing with saline. A Luer lock cap is then attached to the needle to prevent the escape of the saline, which serves to keep blood from entering the plastic tubing while it is being inserted

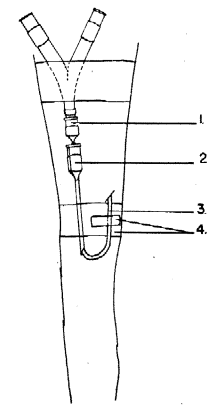

FIG. 4. Arrangement of Y-tube and plastic tubing during injection of drug into radial vein: 1, 23-gauge needle with Y-tube replacing Luer lock cap; 2, 16-gauge needle withdrawn from vein but left on tubing; 3, plastic tubing in vein and taped in place; 4, adhesive tape.

into the vein. The plastic tubing is then passed through the needle into the vein (Fig. 3). The extravenous part of the plastic tubing is bent upward to form a U, so that the needle in the tubing can be attached to the stem of the Y-tube (Fig. 4). The 16-gauge needle, after being withdrawn from the vein, is left on the plastic tubing because the 23-gauge needle prevents its removal. When the radial vein is used, a longer piece of tubing is required to connect the upper end of the drug reservoir to the oil-filled glass tubing. The advantage of using the radial vein is that the plastic tubing is introduced just before the experiment is started and removed at the end of each experiment.

In experiments in process it has been possible to inject 0.2 and 0.4 ml of a 1% solution of acetylcholine chloride repeatedly, without disturbing the dog. With each injection there resulted a short period of inhibition of cardiac activity followed by compensatory acceleration of the heart rate (Fig. 5). With a bell as a conditional stimulus, experiments are being carried out to determine whether the cardiac inhibition or compensatory rise following the injection of acetylcholine as the unconditional stimulus can be condi-

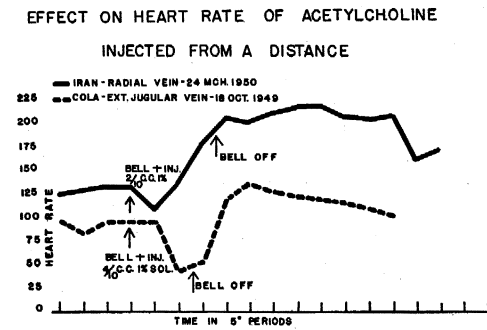

FIG. 5. Effect on heart rate of acetylocholine injected intravenously from a distance. Arrows indicate duration of the bell-ringing used as the conditional stimulus. Drug was injected at the same time bell was started. Injection required several seconds.

tioned. It is planned to study similarly the effects on respiration and salivation, as acetylcholine inhibits respiration and causes increased secretion of saliva. Other related humoral agents will also be investigated.

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A Suggested Biosynthesis of Cyclopropane Rings

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An unusual fatty acid was recently reported by Hofmann and Lucas (*1*). In addition to possessing an odd number of carbon atoms, the acid gives chemical reactions which point strongly to the presence of a three-membered ring. The authors formulated the structure in a general way as I.

$$CH_3(CH_2)_xCH{-}CH(CH_2)_yCOOH \quad (\text{bridged by } CH) \qquad x+y=14$$

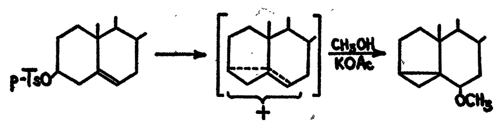
I

The x-ray diffraction pattern indicated a chain length in the 18-carbon range (*1*), an observation consistent with other information on the cyclopropane ring with regard to size and geometry (*2, 3*). Walsh (*2*), among others, has collected a good deal of information on the similarity of the cyclopropane ring to the ordinary double bond, focusing most attention on the availability of electrons (measured by spectra, conjugation, reactivity, etc.). Externally, the cyclopropane ring is analogous to a double bond, although "weaker" as an electron source, or as a conjugating group. Therefore, an organism which was not selective to a high degree would have a difficult time distinguishing between a C_{18} fatty acid and the C_{19} fatty acid containing the cyclopropane ring. The new acid was isolated from the lipid fraction of *Lactobacillus arabinosus,* and the acid may well serve to replace some C_{18} fatty acid.

Cyclopropane rings are a rarity in natural products, and only certain terpenes, the thujanes, caranes, and sabinenes, which contain a three-membered ring as well as a six, occur to any extent (*4*). Since three-membered rings are formed with relative difficulty in the laboratory, the biosynthesis of such rings becomes of interest. Most preparations utilize the displacement of halide by an anion formed by a strong base. Anion formation takes place when the hydrogens are "activated" by a neighboring group such as carbonyl which withdraws electrons. A typical synthesis (*5*) of this type is:

$$Cl{-}CH_2CH_2CH_2CN \xrightarrow{NaNH_2} [Cl{-}CH_2CH_2\ddot{C}H{-}CN] \longrightarrow CH_2{-}CH{-}CN \quad (\text{bridged by } CH_2)$$

One known method of formation is more amenable to biosynthetic conditions. Stoll (*6*) discovered that solvolysis of cholesteryl *p*-toluenesulfonate in methanol buffered with potassium acetate led to the formation of an ether isomeric with cholesteryl methyl ether. Wallis *et al.* (*7*) in this country and Heilbron *et al.* (*8*) in England have elucidated the structure of the product, and Winstein (*9, 10*) has proposed a mechanism to fit the available data. The formation of isomeric (or i-) methyl ether may be formulated as follows:



The reaction is not limited to the sterol series, but no careful work on aliphatic compounds has been performed for the purpose of clarifying the mechanism.

The formation of the cyclopropane ring seems to depend on two related factors: (1) the ionization of a bond, and (2) the presence of a double bond in the proper relation to the ionizing bond. These are connected in the proposed mechanism because the double bond can aid the ionization by supplying electrons. Ionization also depends upon the character of the anion formed, and experience shows that the anions of strong acids (like the *p*-toluenesulfonate ion) are favorable.

With these facts in mind, a biosynthetic route to the acid, I, may be suggested. The precursor must possess the structure II in which *X* is a group which will promote the process of ionization. Ricinoleic acid, III, the only common (*11*) hydroxy-unsaturated fatty acid, fulfills the requirement after the hydroxyl has

$$R{-}C(H)(X){-}CH_2CH{=}CHR'$$

II

$$CH_3(CH_2)_5CH(OH)CH_2CH{=}CH(CH_2)_7COOH$$

III

been converted to the phosphate ester by a phosphorylating enzyme system in a common variety of biochemical transformation. After or during ring closure, reduction would lead to a C_{18} acid; however, this acid would resemble a C_{17} straight chain acid and could conceivably be changed to a C_{19} acid (C_{18} analogue) by some such series of reactions as phosphorylation of the carboxyl group, condensation with acetate, conversion to a 2-ketoacid, and decarbonylation. On the foregoing basis, structure I will appear as IV. A chemical synthesis of IV could be designed with ricinoleic acid as starting material and utilizing the

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[2] The author would like to express his appreciation to S. Winstein for helpful discussion.