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8637 C*

Cyanide Inhibition of Frog Tissues.

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Some time ago it was reported that cyanide produces practically complete inhibition of nerve respiration and that when lower grades of inhibition were observed this was attributable in large part to escape of cyanide into the alkali in the inset.[1] Recently it became desirable to construct a vessel which would permit a study of cyanide inhibition in which preliminary determinations of resting respiration could be carried out in the complete absence of cyanide from the vessel. This was accomplished by the use of a dosing stopcock to contain the cyanide which was placed in the bottom of the vessel so that at any moment by turning this stopcock cyanide would diffuse into the fluid bathing the tissue. When

* C represents a complete, P a preliminary manuscript.

1 Schmitt, F. O., and Schmitt, O. H. A., *Am. J. Physiol.*, 1931, **97**, 302.

cyanide inhibition of frog nerve respiration was studied, not only was the previous report of complete inhibition confirmed, but even very low concentrations of cyanide had considerable effect. Such low concentrations could not be studied with the previous method because of escape of HCN. The inhibitions obtained with other tissues were also higher than those reported by others.

At the outset of the experiment the hollow dosing stopcock, greased and inserted into the female member with the opening facing downwards, is completely filled with neutralized buffered sodium cyanide by means of a capillary pipette. The stopcock is then turned through 90°. The nerves or other tissue to be studied are then placed in the main vessel in 1.0 cc. of phosphate Ringer, 5% KOH being placed in the side arm for absorption of CO_2. The vessels are placed on differential manometers and a curve of resting respiration determined. After obtaining a satisfactory line for resting respiration the dosing stopcocks of the control and experimental vessels are turned so that the opening is now to the vessels permitting diffusion of cyanide into the fluid around the tissue. The usual result is an immediate decrease of respiration which, after 15 to 20 minutes, with concentrations of cyanide greater than about 0.0004 M becomes practically complete inhibition (96-100%). With low concentrations of cyanide the inhibition begins to wear off after an hour or 2 and eventually disappears entirely. There is seldom any tendency for tissues such as nerves to fall through the hole into the dosing stopcock. If desirable a small irregularly shaped glass pearl may be placed over the hole to prevent this in the case of more finely divided tissue.

TABLE I.
Cyanide Inhibition of Respiration of Frog Tissue. T = 19-22° C.

Tissue	Maximum Equilibrium Concentration of NaCN M	Maximum Inhibition %
Nerve	.001	98
"	.0005	99
"	.0002	81
"	.00009	46
"	.00001	27
Kidney	.01	94
"	.005	89
"	.001	79
"	.0005	57
Stomach	.01	94
"	.005	85
Intestine	.001	78

Aside from the fact that with this vessel the resting respiration of the tissue may be first determined in the complete absence of cyanide, there is the further advantage that any escape of cyanide into the alkali of the inset must take place by diffusion through the medium bathing the tissue. The latter point appears to be of considerable importance for, as shown in Table I, with this method the inhibitions obtained with tissues such as kidney, stomach and intestine are considerably higher than those usually reported for these tissues. For example Dixon and Elliott[2] and Muntwyler and Binns[3] obtained a maximum inhibition of 60-80% for these tissues whereas with this method, inhibitions of 85-95% have been obtained regularly with 0.01 to 0.05 M NaCN. In view of the convenience of this method and the considerably higher degree of inhibition obtained with it as compared with the conventional method it has been adopted in this laboratory for all experiments on the effect of cyanide or other volatile reagents upon tissue respiration.

8638 P

Action of Drugs, Nerves and Electric Current in Iris Sphincter.

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It was thought that electrical stimulation might help to locate the site of action of a neural hormone.

A ring or strip a few millimeters wide was cut from the pupillary margin of the iris, usually of a cat, and hung to a writing lever between platinum electrodes in a modified Locke solution of pH 7.4, warmed to about 37°C. The sphincter thus isolated was stimulated electrically, usually for about 5 seconds at a time, with a faradic current from a Harvard inductorium using 3 fresh dry cells in the primary.

The accompanying sample tracing illustrates the findings that:

Electrical stimulation, 1E, of the isolated sphincter muscle caused a contraction followed by a much greater relaxation. This suggests that the electric current "stimulated" first a contractor re-

2 Dixon, M., and Elliott, K. A. C., *Biochem. J.*, 1929, **23**, 812.

3 Muntwyler, E., and Binns, D., *Am. J. Physiol.*, 1934, **108**, 80.