

Non-Specific Effect of Bromide on Conditioned Avoidance Behavior in Rats.* (25463)

IRA ROSENBLUM (Introduced by F. C. Ferguson, Jr.)

Dept. of Pharmacology, Albany Medical College, Albany, N. Y.

Bromide ion has been traditionally classified as a general central nervous system depressant and been used as a sedative and anti-epileptic drug. It has also been reported that bromide possesses ataractic or tranquilizing properties(1), suggesting another therapeutic use for it. Since there is little experimental information about the tranquilizing properties of bromide(2), this experiment examined the effect of bromide on conditioned avoidance response, using it as one criterion of tranquilizing activity(3).

Methods. The avoidance conditioning method described by Cook and Weidley(4) was used with modification. The test animal climbs a vertical pole to avoid unconditioned stimulus (US) (electric shock) delivered through a grid in floor of a box. The conditioning stimulus (CS) is a buzzer introduced for 5 seconds before shock. If animal climbs the pole during this period, no shock is delivered and this becomes the conditioned response (CR). Failure to mount the pole results in shock (3/second), for no more than 30 seconds. If animal climbs the pole during this period, then this becomes the unconditioned response (UR). A specific blockade of CR is indicated by failure to respond to CS though responding to the US. Non-specific blockade results from failure to respond to both CS and US. Male rats of Sprague-Dawley descent, weighing approximately 100 g at start of experiment, were used in groups of 10 for training. Twenty-five rats trained to the CR response were divided randomly into 5 groups. In each group, 1 animal served as control, and the other 4 received 100, 200, 300, 400 or 500 mg/kg respectively of Br⁻ administered as NaBr. Injections were made intraperitoneally, once daily, 5 days a week for 2 weeks to achieve a relatively stable blood Br⁻ level or until blockade of CR was noted.

TABLE I. Effect of Bromide on Conditioned Avoidance Response.

Dose Br ⁻ (mg/kg)	Blood Br ⁻ (meq/l) *	No. rats tested	No. rats fail- ing to respond	
			CR	UR
Control		5	0	0
100	12.0 ± 2.3	4	0	0
200	18.2 ± 2.8	"	0	0
300	27.7 ± 5.3	"	0	0
400	35.0 ± 1.9	"	0	0
500	48.7 ± 1.7	"	4	4

* Mean ± stand. dev.

Testing was carried out each day approximately 4 hours after injection of NaBr. After 2 weeks of testing, or when blockade of CR was noted, a blood sample was taken by cardiac puncture under light ether anesthesia. The sample was analyzed for whole blood Br⁻ by the method of Brodie and Friedman(5).

Results. A wide range of blood Br⁻ levels were obtained (Table I) with group mean values ranging from 12 to 48.7 meq/l. At highest level of blood Br⁻ the CR was blocked. However this blockade was non-specific since the UR was also blocked. At this blood Br⁻ level all rats showed evidence of ataxia and loss of righting reflex, accounting for inability to respond to the US by climbing the pole. This type of blockade of both CR and UR occurs with administration of such general central nervous depressants as barbital and methylparafynol(4). Therefore, it seems that the effect of Br⁻ on the CR is similar to that of a general central nervous depressant rather than to that of a drug possessing specific tranquilizing activity.

Although only one method for assessing tranquilizing activity of drugs was employed, the same relationship between toxic and effective dose also occurs when Br⁻ is tested for ability to block audiogenic seizures in rats(6). In these tests a dose of 750 mg/kg of NaBr given intraperitoneally to rats was 100% successful in preventing seizures but caused incoordination in the animals. Since ability to

* Supported by grant from Nat. Inst. Health, U.S.P.H.S.

block audiogenic seizures has also been used to assess tranquilizing activity of drugs(3), it lends further support to the assumption that Br^- does not possess tranquilizing properties in non-toxic doses.

Summary. The effect of 5 doses of bromide was tested for effect on conditioned avoidance response in rats. Blockade of response occurred at dose of 500 mg/kg of Br^- , but this effect was non-specific, since it was accompanied by ataxia and loss of righting reflex.

1. Sperling, F., *Fed. Proc.*, 1957, v16, 337.
2. Jacobsen, E., *J. Pharm., Lond.*, 1958, v10, 273.
3. Riley, H., Spink, A., *ibid.*, 657.
4. Cock, L., Weidley, E., *Ann. N. Y. Acad. Sci.*, 1957, v66, 740.
5. Brodie, B. B., Friedman, M. M., *J. Biol. Chem.*, 1938, v124, 511.
6. Corn, M., Lester, D., Greenberg, L. A., *J. Pharm. Exp. Ther.*, 1955, v133, 58.

Received October 2, 1959. P.S.E.B.M., 1959, v103.

Inheritance of Capability of Human Esophageal Epithelial Cells to Grow with Diverse Carbohydrates.* (25464)

S. GAYLEN BRADLEY AND JEROME T. SYVERTON†

Dept. of Bacteriology and Immunology, University of Minnesota, Minneapolis

Several carbohydrates have been reported sufficient for growth of human cells in continuous culture in a medium composed of inorganic salts and dialyzed serum(1,2). Additionally, Chang(3) derived variants of HeLa and conjunctival cells able to grow in medium containing d+xylose, a usually insufficient sugar, as sole carbon-energy source. Previous work has not determined whether such capacity of human cells to grow with particular carbohydrates is constitutive or adaptive, or how such capacity is inherited. This paper is concerned with capacity of cells in culture to initiate and continue multiplication, given particular carbohydrates. Capacity of cultures to grow in particular carbohydrate media was employed as a measure of cell capacity. This measure was influenced by the proportion of capable or potentially capable cells in culture inocula, degree of capability of such cells, and ability of these cells to become established in surface culture. When capability to grow in a medium containing a specific carbohydrate was independent of continued addition of that carbohy-

drate during subculture, the capability was considered hereditary. If capability to grow in a particular medium was attenuated reversibly by substitution of another carbohydrate during subculture, the capability was considered adaptive, as opposed to constitutive. By definition, the presence of a constitutive enzyme and the power to make an adaptive enzyme are hereditary. The expression of an adaptive enzyme is dependent upon the substrate and therefore nonhereditary. Irreversible alteration of culture capability by presentation or withdrawal of the carbohydrate was viewed as evidence of selective enrichment of a culture with capable or incapable cells. This report is concerned with propagation of human esophageal epithelial cells in medium with various carbohydrates, derivation of substrains by serial culture in media containing fructose, d+xylose or d+galactose, and determination of heritability of capacity to grow in these carbohydrate media.

Materials and methods. Cells of human esophageal epithelium (Minn. EE 55-12-1) have been carried as a stable line for 4 years after establishment from tissue of a male infant with tracheo-esophageal fistula(4). **Medium** for growth of the parental (uncloned) EE strain contained 105 mM sodium chloride, 5 mM potassium chloride, 0.8 mM disodium

* Aided by grant from Nat. Foundation, Nat. Cancer Inst. and Frederica Beard Memorial Grant for Cancer Research, Am. Cancer Soc.

† The authors acknowledge valuable technical assistance of Lily A. Jones.