Whether Bovine #10 is identical with a human adenovirus or an antigenic relative remains to be determined. If it is identical with a human virus we may have identified an important reservoir of these agents, for the very high incidence of infection in cattle indicates the agents are endemic in these aninals. We are inclined to believe, however, that Bovine #10 is antigenically related to a human virus, in the sense of a cowpox-smallpox relationship, since we have not been able to achieve one characteristic of human adenoviruses, namely growth in HeLa cells. There is of course a vaccine potential if this latter relationship is the proper one.

We believe that isolation of an adenovirus from cattle gives added weight to the implication, based on neutralizing antibodies to polioviruses in calf sera, that cattle may harbor polioviruses or their antigenic relatives. We may note in passing that 45% of bacterial, fungal, and parasitic infections of animals are shared by man, and if we accept this figure as one that will be valid for viruses we must conclude that a large number of human viruses or their antigenic relatives will ultimately be isolated from animals.

*Summary.* A virus has been isolated from feces of an apparently normal cow that has been identified as an adenovirus related to or identical with an as yet unidentified type of human adenovirus.

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## Effect of Compound 48/80 on Toxicity of Histamine and of Serotonin in Mice.* (25124)

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Tissue mast cells rapidly respond to various types of injurious stimuli by shedding their cytoplasmic contents (*i.e.*, granules) into the surrounding ground substance of the connective tissue. Since these cells contain relatively large concentrations of both histamine and heparin and, in the mouse and rat, also serotonin (5-hydroxytryptamine)(1,2,3), release of these biologically-active substances undoubtedly influences the nature of subsequent responses of the local tissue to the injurious stimulus. Degranulation of mast cells can be readily initiated by a synthetic phenyl alkylamine substance called compound 48/80 (4). This drug is a potent releaser of histamine(5), has a protamine-like effect on heparin(4) and, in mouse and rat, will also release serotonin(6,7). In addition, it produces a lethal intoxication in the mouse and various other laboratory animals(8). Although Riley (9), and others(5,10), suggested that pharmacologic or injurious effects of compound 48/80 are mediated by the histamine and/or 5-hydroxytryptamine released from mast cells, it would seem unlikely that such a mechanism could account for *lethal* effects of this drug in the mouse. In the first place, tissue levels of histamine and serotonin in the mouse(11) are many times less than those required for lethal effects of these amines in this species (12,13). Secondly, the interaction of compound 48/80 with mast cells should result in

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binding of this drug by heparin with a concomitant reduction in its lethal effects(14). The following report is concerned with the relative importance of mast cell constituents (*i.e.*, histamine, serotonin and heparin) in lethal intoxication produced by compound 48/80 in the mouse.

*Materials and methods.* CBA mice, mixed sexes, 2 to 3 months of age and ranging in weight from 19 to 21 g were used. Drugs used were: histamine dihydrochloride, histidine chloride, 5-hydroxytryptamine creatinine sulphate and tryptamine chloride obtained from Nutritional Biochemical Corp. Amounts used are given in terms of the active constituent. Compound 48/80‡ was obtained from Burroughs Wellcome & Co., and Na heparinate (117 $\mu$/mg)‡ from Upjohn Co. All drugs were prepared for injection in isotonic, pyrogen-free, sterile saline and administered either singly or in combination in 0.25 ml to the mice *via* tail vein. Intravenous route was used since it is the most sensitive means for detecting lethal effects of histamine, serotonin or compound 48/80. To evaluate toxicity of each of the test drugs, or combinations of drugs, a minimum of 10 animals was employed for each test group and determinations made on the basis of numbers of animals surviving 24 hours after challenge. Results of titrations are presented as 50% lethal doses or 50% protective doses (heparin) as determined by the method of Reed and Muench(15).

*Results. Histamine and serotonin.* Preliminary experiments were performed by injecting control groups of mice with relatively small doses of either histamine or serotonin or their analogues, histidine or tryptamine, respectively. Comparable groups of test animals were injected with equivalent doses of test substances to each of which had been added a standard sublethal amount of compound 48/80 (2.0 $\mu$g/g). Histamine was markedly toxic in presence of compound 48/80, whereas histidine at comparable dose levels was not (Table I). Likewise, the effect of compound 48/80 on toxicity of serotonin was far more marked than on tryptamine. This toxic effect could also be demonstrated if compound 48/80 and amine were given separately but was markedly decreased if time interval between doses was greater than 15 minutes.

TABLE I. Effect of Compound 48/80 on Toxicity of Histamine, 5-OH Tryptamine and Related Substances.

| Test drugs ($\mu$g/g) | Survival ratios (S/T)* Saline controls | Plus compound 48/80 (2 $\mu$g/g) |
|---|---|---|
| Saline | | 10/10 |
| Histamine (60) | 10/10 | 1/10 |
| " (80) | " | 10/10 |
| 5-OH tryptamine (1) | " | 0/10 |
| Tryptamine (4) | " | 9/10 |

* Groups of mice challenged by intrav. route with 0.25 ml saline containing test drug alone or in combination with standard sublethal dose of compound 48/80.

A dose relationship was determined between compound 48/80 and the amines, histamine and serotonin. A series of titrations were performed in each of which groups of mice were challenged with graded doses of one of the amines to which had been added one of a number of standard sublethal amounts of compound 48/80 (0, 1, 1.5 or 2 $\mu$g/g). Amount of amine required for a lethal dose effect was determined for each compound 48/80 dose levels. Results are shown in Fig. 1 in which each standard sublethal dose of compound 48/80 (abscissa) are plotted against respective complementary amount of amine (free base) required for an $LD_{50}$ effect. With histamine, the points approximate a straight line drawn between $LD_{50}$ doses of histamine and of compound 48/80. This would suggest that lethal effects of compound 48/80 and histamine are additive. The compound 48/80-serotonin relationship proved to be somewhat different with remarkably small amounts of serotonin being required for $LD_{50}$ effect in compound 48/80 dose range of 1 to 2 $\mu$g/g. This would indicate that compound 48/80 synergizes serotonin toxicity.

Results were 166, 103, 56.6, 26.5 and .0 $\mu$g histamine base/g body wt. and 137.4, 26.5, 4.2, .4 and .0 $\mu$g serotonin base/g body wt. for $LD_{50}$ effects at compound 48/80 dose levels of .0, 1, 1.5, 2 and 2.2 $\mu$g/g, respectively.

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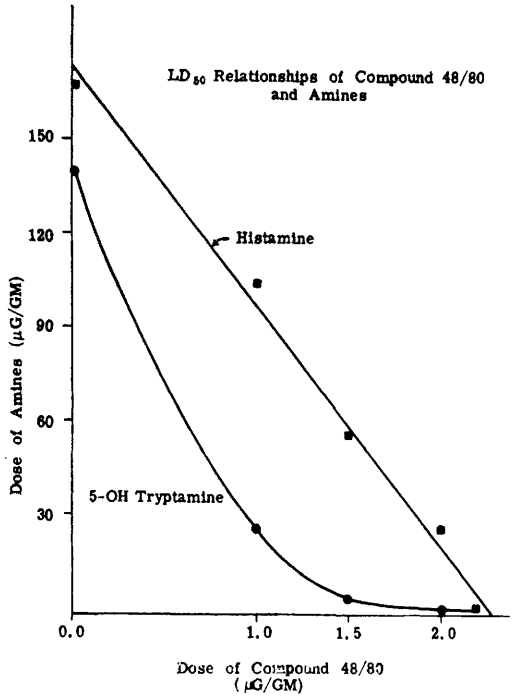


FIG. 1. $LD_{50}$ relationships of compound 48/80 with histamine or 5-hydroxytryptamine in mice. Individual $LD_{50}$ results obtained following intrav. challenge of groups of mice (10 animals in each) with specific doses of compound 48/80 (abscissa) containing graded amounts of one of the amines. Individual $LD_{50}$ doses of either histamine or 5-hydroxytryptamine are many times greater than that of compound 48/80.

When these data are compared to tissue levels of histamine (8 - 10 μg/g)(12,11) and of serotonin (.2 - 2 μg/g)(16,11) reported for the mouse, it becomes evident that the apparent additive or synergistic effects of compound 48/80 on amine toxicity could not be directly due to supplementation of the exogenously administered amines by those released from the tissues. In this regard, it has been observed that serotonin (70 μg/g) did not elevate the sublethal effect of histamine (60 μg/g) to a lethal dose. The lethal effects of compound 48/80 therefore appear to result from a primary toxic effect on tissues which are not solely dependent on release of these amines. The markedly reduced tolerance of mice for either histamine or serotonin in presence of compound 48/80 is considered as evidence of this tissue injury.

*Heparin.* It has been previously suggested that release of heparin from mast cells in their response to compound 48/80 can be of salutary value in that this acidic mucopolysaccharide will protect against the lethal effects of this cationic drug(14). The relative value of the protective effect of heparin versus compound 48/80-enhancing effect on amine toxicity was investigated. A series of titrations were performed with heparin *versus* standard minimal (100%) lethal doses of histamine, serotonin, mixtures of compound 48/80 with the respective amines or compound 48/80 alone. This was performed by injecting groups of mice with one of the lethal challenge doses to which had been added one of a series of graded amounts of heparin. In this way, 50% protective dose of heparin was determined for each group in the series of challenges shown in Table II.

Heparin, in doses to 200 μg/g, gave no evidence of protection against either histamine or serotonin and only partial protection against 1 μg/g compound 48/80 plus 135 μg/g histamine. Protection in this latter group varied between 30 and 40% with doses of heparin in the range of 50 to 200 μg/g. However, in all other groups only relatively small amounts of heparin were required for complete protection against lethal doses of compound 48/80-histamine, compound 48/80-serotonin or compound 48/80 alone. Heparin protection appeared related to compound 48/80 content of the challenge dose with the ratios of 50% protective doses of heparin to their respective compound 48/80 doses being somewhat variable but less than 1 in the histamine group and approximating 0.2 in the serotonin group. It thus appears that direct interaction of heparin with compound 48/80 can indirectly affect toxicity of the respective amines.

Lethal effects of compound 48/80-amine mixtures are dependent on relative amounts of both constituents (Fig. 1). In each instance of heparin protection versus compound 48/80-enhanced intoxication with either histamine or serotonin, the amount of heparin required was much less than the amount of amine employed (Table II). It was also observed that with a given dose of compound 48/80, the amount of heparin required varied directly with amount of amine involved. If

TABLE II. Effect of Heparin on Compound 48/80-Enhanced Toxicity of Histamine and 5-Hydroxytryptamine.

| Compound 48/80 ($\mu$g/g) | A. Histamine | | | B. 5-OH tryptamine | | |
|---|---|---|---|---|---|---|
| | Dose ($\mu$g/g) | Heparin $PD_{50}$ ($\mu$g/g) | Heparin: Cmpd. 48/80 | Dose ($\mu$g/g) | Heparin $PD_{50}$ ($\mu$g/g) | Heparin: Cmpd. 48/80 |
| .0 | 377.0 | 200.0 | | 160.0 | 200.0 | |
| 1.0 | 135.0 | 50.0* | | 34.3 | .22 | .22 |
| 1.5 | 90.0 | 1.3 | .87 | 10.1 | .21 | .14 |
| 2.0 | 30.0 | .35 | .18 | .92 | .4 | .2 |
| 2.67 | .0 | .7 | .27 | .0 | .7 | .27 |

Groups of at least 10 mice each were challenged by intrav. route with 0.25 ml saline containing one of standard mixtures (minimal lethal dose) of compound 48/80 with either (A) histamine or (B) 5-hydroxytryptamine plus one of a series of graded amounts of heparin. Results are presented in terms of 50% protective dose of heparin ($PD_{50}$). The ratios of these heparin doses to appropriate compound 48/80 doses are also shown.

* % survival did not exceed 40% in heparin dose range of 50 to 200 $\mu$g/g.

these relationships could be considered valid for interaction of compound 48/80 with the mast cell, then the contribution of the mast cell to the lethal effects of a given dose of compound 48/80 would depend on the relative amounts of histamine and/or serotonin to heparin content of this cell. Since the literature(17) shows the mast cell to have a smaller amine content (approx. 1% histamine and possibly 0.1% serotonin) than it does heparin (approx. 3%), the exclusive interaction of a given dose of compound 48/80 with mast cells would be more likely to inhibit than to mediate the lethal effects produced by this drug.

*Discussion.* The biological significance of mast cell degranulation in response to compound 48/80 has been generally related to *release* of histamine and, in some species, of serotonin and to subsequent pharmacologic effects of these amines(5,7,9,10). Although neither histamine nor serotonin is present in tissues of the mouse in sufficient amounts to account directly for the lethal effects of compound 48/80, it was observed that compound 48/80 could markedly increase the lethal effects of these amines in the mouse. Compound 48/80 would thus appear to have a primary lethal effect quite distinct from alterations in the physiologic state of tissues which are induced by the released amines. This point is well supported by the observations of others(8,18,19,20). Since this injurious effect of compound 48/80 can be manifested as a decreased resistance to histamine and to serotonin, the release of these amines may be contributory to the total lethal effect but would not otherwise appear to be of major importance in this regard. Moreover, it has been observed in studies to be reported later, that prior treatment of mice with multiple sublethal doses of either compound 48/80 or polymyxin B to "deplete" their tissues of histamine and serotonin, does not provide any greater degree of resistance to the lethal effects of an *i.v.* challenge with compound 48/80 than can be obtained simply by pretreatment of animals with saline.

As discussed elsewhere(11,21), release of histamine by compound 48/80 is probably the result of an ion exchange in which heparin, serving as a cation exchanger, releases histamine in exchange for compound 48/80. This would result in a reduction in amount of compound 48/80 available to interact at more vital sites in the tissues. Since this drug is many times more toxic than is histamine (or serotonin), this exchange reaction can be of marked benefit to the organism. In this regard, heparin treatment will readily protect mice against lethal effects of compound 48/80 (14). The reaction of mast cell to compound 48/80 has been considered as essentially a protective response of connective tissue to injury and the associated release of histamine and serotonin as primarily a manifestation of this response. The degree to which the released amines may then participate in the lethal effects of this drug would be conditioned by amount of injury directly produced by compound 48/80.

*Summary.* (1) Compound 48/80 appears to have an additive effect on toxicity of hista-

mine and a synergistic effect on toxicity of serotonin in the mouse. (2) Heparin interferes with this compound 48/80 effect on lethal intoxication with histamine or serotonin by direct interaction with compound 48/80 but does not appear to alter individual toxicities of respective amines. (3) It is suggested that degranulation of mast cells in response to compound 48/80 is a protective function of connective tissue in which the releaser drug is sequestered by heparin of the mast cell. The associated release of amines is thought to be a manifestation of this interaction between mast cells and compound 48/80 and only indirectly related (if at all) to lethal effects of this drug.

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## Rapid Sensitive Method for Determining $H^3$-Water in Body Fluids by Liquid Scintillation Spectrometry.* (25125)

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The assay of tritium water by liquid scintillation spectrometry is difficult for 2 main reasons: a) water is not efficient in transferring the excitation energy of tritium radiation to the phosphor; and b) water is insoluble in the known aromatic solvents (toluene and xylene) which are most effective in making this transfer. In the methods of Kinard (1), Hayes and Gould(2), and Okita *et al.* (3), alcohol is used to solubilize water with xylene or toluene. This results in considerable dilution of the $H^3$ in the water sample, thus limiting these methods to samples of tritium water with high radioactivity. Furst *et al.* (4) were the first to report that naphthalene can restore, to a large extent, the counting efficiency lost by addition of water to dioxane solutions containing tritium. This observation formed the basis of the more efficient assay developed by Langham *et al.*(5). A method for increasing still further the efficiency of counting tritium water in biological fluids is described here. Some of the conditions that influence the efficiency of the assay of tritiated water by the dioxane-naphthalene

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