LOSS OF BIOLOGICAL ACTIVITY OF APOMORPHINE FROM AUTO-OXIDATION

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Buffered and unbuffered solutions of apomorphine hydrochloride in physiological saline were incubated at 30° for 61 days and samples were periodically withdrawn for both chemical and biological assay. Within the limits of error, the loss of biological activity, as measured by the gradual diminution in the intensity of the pecking syndrome in pigeons, paralleled the disappearance of unoxidised apomorphine. The un-buffered solutions retained approximately 75 per cent of their biological activity after 60 days incubation while solutions buffered initially to pH 6·0 were biologically inert after 16 days. Extracted and concentrated degradation product(s) when administered to pigeons failed to initiate the pecking syndrome.

THE development of a readily quantifiable measure of pecking syndrome intensity in pigeons provides us with a useful and reliable index of apomorphine potency. From an experimental point of view, it has proven to be more reliable than those methods utilising the familiar emetic response in dogs. Using the pigeon assay we have been able to effectively monitor apomorphine activity under a variety of experimental conditions and have adapted the method as a screening procedure for potential anti-emetic, psycholeptic agents (Burkman, 1961a, 1962). The applicability of such a method is based upon an apparent parallelism existing between the avian response and emesis in mammals.

The recent investigations of apomorphine by Kaul, Brochmann-Hanssen and Way (1961a,b,c), have further stimulated interest in the activity of apomorphine metabolites as pecking syndrome stimulants. The present communication describes a study undertaken to determine whether the products of spontaneous oxidation retain the ability to initiate the avian syndrome.

EXPERIMENTAL METHODS

Solutions. Unbuffered solutions of 100 ml. quantities of 0·9 per cent sodium chloride contained 50 mg. of apomorphine hydrochloride. Buffered solutions were prepared by dissolving 50 mg. of apomorphine hydrochloride in 50 ml. of 0·9 per cent sodium chloride, adding 4 ml. of buffer, pH 6·0 (McIlvaine, 1921) and sufficient saline to make 100 ml. Four preparations of both buffered and unbuffered solutions served as experimental replicates.

Incubation. Solutions were placed in 250 ml. glass stoppered Erlenmeyer flasks immersed to the neck in a water bath maintained at 30 $\pm$ 0·1°. The flasks were charged daily with oxygen and subjected to intermittent agitation to discourage adhesion of degradation products to the inner walls.

Determination of unoxidised apomorphine. The spectrophotometric method of Kaul, Brochmann-Hanssen and Way (1959) was used. The only changes made were in the volumes of extraction solvents and buffer used to better accommodate the quantities of apomorphine being assayed. Samples from freshly prepared solutions, assayed at zero incubation time, served as controls. The results from subsequent determinations were expressed as per cent of their control. Thus, the disappearance of apomorphine served as the measure of chemical degradation.

Biological assay. Groups of adult domestic pigeons (*Columba livia*) of mixed sex were initially screened for apomorphine sensitivity at the 0·5 mg./kg. level and resistant animals discarded. Four birds served as assay subjects for *each* of the incubated preparations of apomorphine (a total of 32 pigeons). Samples of solutions were periodically removed and administered to birds in doses of 0·5 mg./kg., assuming a constant concentration of 0·5 mg. of apomorphine hydrochloride per ml. throughout the incubation period. The cumulative pecking responses evoked by the freshly prepared solutions (zero incubation time) represented control values against which subsequent measurements were compared. The results were expressed as per cent of control.

Quantitative assay of the pecking syndrome has been described elsewhere (Burkman, 1961b). All injections were made into the peritoneal cavity.

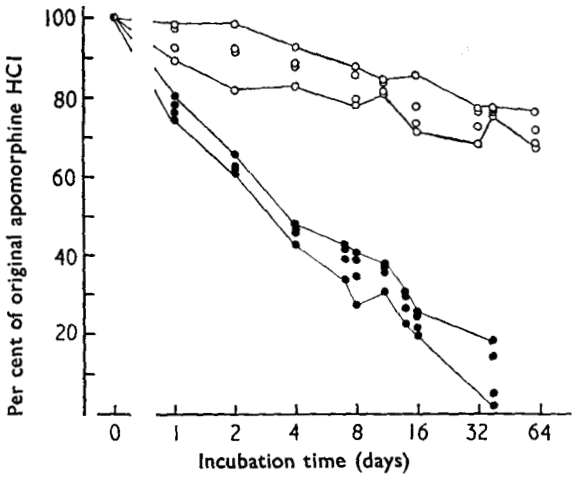

FIG. 1. Apomorphine HCl degradation at 30°. Original concentration: 0·5 mg./ml. of 0·9 per cent sodium chloride solution. —○— Unbuffered (Initial pH 5·5). —●— Buffered initially to pH 6·0.

Extractions of degradation product(s). Oxidised solutions, originally containing 0·5 mg./ml. of apomorphine hydrochloride in 0·9 per cent sodium chloride, were extracted with chloroform and the chloroform phase washed several times with N hydrochloric acid. The blue-black chloroform fraction was then washed repeatedly with water, dried over anhydrous sodium sulphate and the solvent removed under reduced

pressure as recommended by Kaul and others (1961d). The chloroform soluble residue* was suspended in water containing 2 per cent methylcellulose (1,500 cps). This suspension was intraperitoneally administered to pigeons in varying doses and the birds were observed for the following 6 hr.

Results and Discussion

The alterations in concentration of apomorphine hydrochloride and the corresponding changes in biological activity of the preparations with time are graphically presented in Figs. 1 and 2. The higher pH favours more rapid degradation as has been demonstrated by Veit (1935) and Kaul and others (1959).

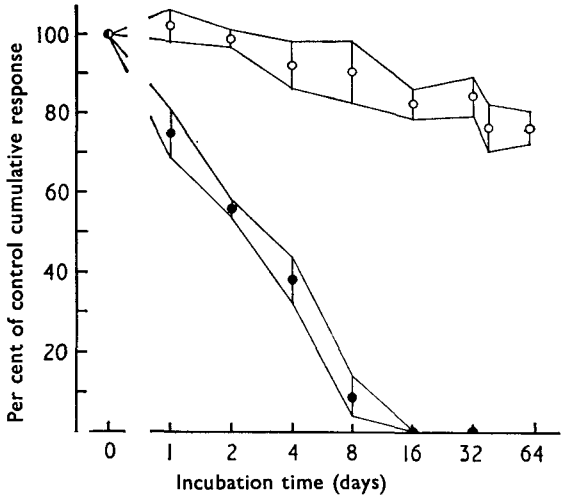

FIG. 2. Biological activity of degrading apomorphine HCl in pigeons. Original concentration: 0·5 mg. per ml. of 0·9 per cent sodium chloride solution. Points represent mean Cumulative Pecking Response ± standard error in terms of per cent of control. —○— Unbuffered (Initial pH 5·5). —●— Buffered initially to pH 6·0.

During the incubation period a gradual reduction in pH of all degrading solutions was noted. The unbuffered preparations had an initial reaction of pH 5·5 which progressively decreased to pH 3·6 after 61 days. Other preparations, buffered initially to pH 6·0, had a reaction of pH 4·6 after 32 days, the most rapid decrease occurring during the last 16 days.

The change in intensity of the pecking syndrome that accompanies the ageing of unbuffered incubated solutions is far from striking and reflects the very gradual inactivation of apomorphine. At the end of 61 days incubation, approximately 70 per cent of the original apomorphine was still present and this was sufficient to initiate a pecking syndrome that was 75 per cent as intense as that produced by the original solution. The

* 50 mg. of apomorphine hydrochloride (42·7 mg. apomorphine base) yielded 25 mg. of chloroform soluble residue.

solution, at this point, was dark green in colour with black precipitate detectable on the floor and walls of the container. Visual inspection entirely fails to give the observer a basis for estimating the degree of decomposition. The very rapidly developing green colour of a freshly prepared unbuffered solution of apomorphine hydrochloride deceptively impresses one with what is interpreted to be a high rate of degradation. It came perhaps as a surprise to Gorrell and Gray (1928) and others (Corbelli, 1911; Ponte, 1935) to discover that although their aged apomorphine preparations were extremely discoloured, they nevertheless still retained emetic activity. On the other hand, there have been reports of complete loss of emetic activity upon storage for 6 months, after sterilising in a water-bath (Laurino, 1936) and after incubation with rabbit serum for 2 hr. (Takahashi, 1934). In none of the studies cited was a quantitative analysis performed and we therefore have no information about the actual state of decomposition. These reports on biological activity changes have been based entirely upon an emetic end-point, a response which is difficult to make quantitative. Thus, statements reflect extremes only—retention or loss of emetic activity with no indication that there may have been a partial loss. Emesis simply does not lend itself readily to a graded quantitative analysis.

Our experience with the apomorphine-induced pecking syndrome has served to provide us with a readily measurable index of apomorphine activity. Initial explorations with aged unbuffered solutions seemed to support the observations of those investigators who failed to detect a loss of apomorphine potency in spite of the fact that the solutions were extremely discoloured. This suggested that either the degradation products possess the same activity as the parent apomorphine or that the intense discoloration may actually be due to the degradation of only minute amounts of apomorphine. The results presented here support the latter explanation. The more rapidly oxidising solutions (buffered initially at pH 6·0) lost activity much faster than unbuffered solutions. In both types of preparations the loss in biological activity closely paralleled the disappearance of unoxidised apomorphine.

Changes in the concentration of apomorphine in the unbuffered solutions occurring during the first 2 days were too small to be detected by the biological method hence changes in activity here are not significant. On the other hand, after 8 days incubation no activity at all could be detected in the buffered preparations since the residual concentration of apomorphine at this time was apparently subthreshold (Burkman, 1960). For these reasons the curves for biological activity cannot be perfectly superimposed over those measuring apomorphine concentration. Within the range of maximal biological sensitivity, however, the two assays are in excellent agreement.

The chloroform-soluble component of the degradation mixture (which represented approximately 45 per cent of the total oxidation product) was administered to a total of 24 pigeons in doses ranging from 0·5 mg./kg. to 10 mg./kg., i.p. All doses failed to evoke a pecking response. The material was, in fact, devoid of all gross behavioural effect.

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