Absorption and Elimination Profile of Isoproterenol II

Parenteral, Oral, and Rectal Administration to Unanesthetized Dogs

By G. A. PORTMANN, H. MINATOYA, and A. M. LANDS

The tachycardia in unanesthetized dogs after parenteral, oral, and rectal administration of isoproterenol in solution has been monitored. Disappearance of heart rate activity after intravenous administration appears to be first order and ranges in half-life from 0.9 to 1.4 min. Comparison of areas under curves indicate the total response after oral administration is proportionately greater as the dose is decreased or given in divided doses. Rectal administration is more efficient than oral dosage. A method for calculating apparent absorption rate constants for drugs having rapid elimination rates relative to slow absorption rates is described and applied to isoproterenol.

STUDIES OF isoproterenol effect on heart rate from different sites in the anesthetized dog have shown some interesting and unique drug properties (1). The extremely rapid disappearance of activity after the cessation of intravenous infusions and the prolonged duration of activity after drug administration in the trachea, small intestine, proximal colon, and rectum suggested that a more detailed study subjected to kinetic analyses would be valuable. This communication presents results obtained in a group of trained dogs used without anesthesia.

MATERIALS AND METHODS

Solutions of isoproterenol, 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol HCl, were prepared fresh in 5% dextrose solution containing sodium bisulfite (1:100,000) and ethylenediaminetetraacetic acid trisodium salt (1:10,000). All doses of isoproterenol are expressed in terms of the base. Nine trained dogs of both sexes, weighing 11–16 Kg. (average weight, 13.6 Kg.), were used repeatedly throughout this investigation. No dog was used more frequently than once a week. During the experimental sessions, the dogs were placed in sling-stand frames designed to keep the animals still but with a minimum of restraint. Heart rate was monitored by means of an electrocardiograph (Viso-Cardiette, Sanborn) using the Lead I attachments. Food was withheld 17–18 hr. prior to medication.

Intravenous injection or infusion was carried out through a fine polyethylene catheter (PE 50) that had been inserted previously into the left saphenous vein. Intravenous injection was either rapid or by a timed 1-min. infusion, the drug dose being contained in 0.25–1.0 ml. volume. The dosage schedule was arranged in a Latin-square design, including a sham medication. Slow intravenous infusion was carried out by means of a pump. Following a control infusion of the vehicle alone for 15 min., isoproterenol infusion was carried out at rates of 0.01–0.08 mcg /Kg./min. for 30 min. The heart rate was monitored at 1–5 min. intervals throughout the infusion and recovery period.

Isoproterenol, in aqueous solution, was administered orally in single or divided doses. The medications were introduced into the back of the mouth with a syringe and followed by a water rinse of 5 ml. Premedication heart rate was determined at intervals of 10 min. for 30 min. Following medication, the rate was determined at 5-min. intervals for 30 min. and at 10-min. intervals thereafter until recovery.

Isoproterenol, in aqueous solution, was instilled rectally to the unanesthetized dog, the dose administered being contained in 0.25 ml. Heart rate changes were followed as described above.

RESULTS

Intravenous Injection.—Doses of 1.25, 2.50, and 5.0 mcg. caused a prompt increase in rate with the maximum at 0.5 min. after the rapid injection and

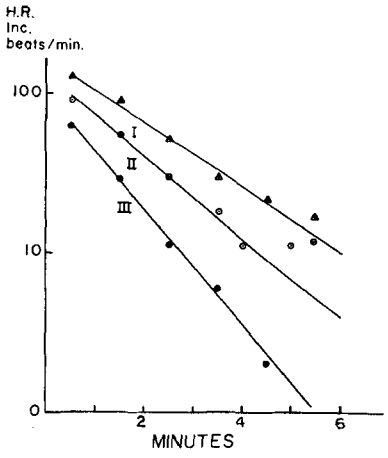

Fig. 1.—Heart rate increase of dogs to the rapid intravenous injection of isoproterenol. Mean responses from nine trained dogs in each instance minus sham injection responses. Injection of I, 1.25 mcg.; II, 2.50 mcg.; and III, 5.0 mcg. drug, as base.

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TABLE I.—ESTIMATE OF THE DISAPPEARANCE RATE OF ISOPROTERENOL IN DOGS FOLLOWING INTRAVENOUS ADMINISTRATION

Dose,[a] mcg.	Rapid Injection,[b] Max. Heart Rate Increase Mean Beats/min. ± S.E.	Half-Life, min.	1-min. Infusion,[c] Max. Heart Rate Increase Mean Beats/min. ± S.E.	Half-Life, min.
1.25	72 ± 11	0.90	63 ± 11	1.10
2.50	100 ± 17	1.20	96 ± 7	0.95
5.00	137 ± 19	1.40	132 ± 6	1.10
Glucose 5% 1.0 ml.	10 ± 4			

[a] Total dose, mcg. base. [b] Data obtained from eight trained dogs, weighing 11–16 Kg. (av. 13.6 Kg.). [c] Data obtained from nine trained dogs.

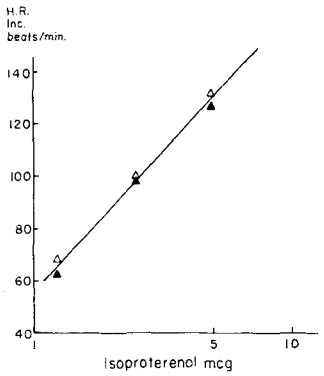


Fig. 2.—Maximum heart rate increase in dogs after intravenous administration by 1-min. infusion (▲) compared with that for rapid intravenous injection (△).

1.5 min. after the slow injection. Rather than evaluate the data from each dog separately, it was chosen to use all of the dogs as a group using mean response values and total dose rather than on a dose per unit weight basis. Therefore, relationship between dose of active drug and cardiac response will be considered independent of the dog weight. Mean values for heart rate changes *versus* time after rapid injection are shown in Fig. 1. These results indicate greater cardiac sensitivity than that found in anesthetized (morphine–pentobarbital) dogs, as previously described (1).

Disappearance half-life estimates from the activity decay curves are listed in Table I as well as the maximum heart rate responses and standard deviations. Average half-life from both injections is 1.11 ± 0.07 min. corresponding to an apparent rate constant of 0.625 min.$^{-1}$. Results after rapid injection suggest a dose-dependent half-life not apparent in the 1-min. infusions (slow injection). The extremely short half-life does not permit the distributive and elimination phases to be distinguished separately.

By determining activity disappearance half-life graphically for each of six dogs after the 5-mcg. dose (rapid injection), the mean value is 1.23 ± 0.16 min., indicating good correlation with the half-life (1.40 min.) obtained using mean heart activity values. This apparent first-order rapid activity decay correlates closely with values reported for epinephrine and norepinephrine in humans (2–4) and in the dog (5–8) where blood concentrations or activities were measured as a function of time. Log dose-response relationship, as illustrated in Fig. 2, indicates close agreement between the two intravenous injection studies. As expected, maximum heart rate responses for the 1-min. infusions at each point appear less than those for rapid injections, although this difference is not significant.

Intravenous Infusion.—Isoproterenol was administered by slow (30 min.) intravenous infusion to the group of dogs described under *Intravenous Injection*. Infusion rates of 0.01–0.08 mcg. (base)/Kg./min. were well tolerated, and the resultant cardiac responses were closely dose dependent. A plot of the steady-state values *versus* dose (logarithmic scale) provided a linear regression (Fig. 3). Upon terminating infusion, drug disappearance, as indicated by heart rate, was very similar to that observed after intravenous injection.

Teorell derived the following equation to describe the changes in concentration for infused drugs (9).

$$Y = \frac{r}{k}(1 - e^{-kt}) \qquad \text{(Eq. 1)}$$

where Y is the quantity of drug in the body, k is the disappearance constant, r is the rate of infusion, and t is a time during infusion.

Estimates of isoproterenol quantities in the body were calculated using the apparent activity decay constant of 0.625 min.$^{-1}$ for k in place of a concentration disappearance constant as defined by Teorell. In view of the small differences in these two constants for epinephrine and norepinephrine as stated previously, the isoproterenol heart activity decay constant is probably a good estimate. At 30 min., which is the steady-state time period for all of the infusion rates, the exponential term in Eq. 1 becomes very small and can be omitted producing

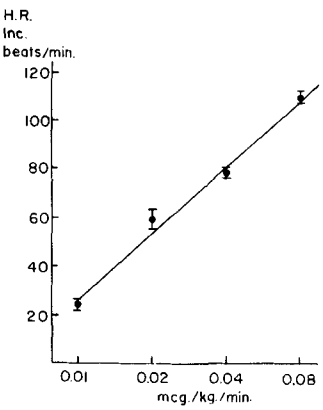


Fig. 3.—Heart rate increase in dogs induced by 30-min. intravenous infusion of isoproterenol. The mean steady-state rates (with standard errors) are shown.

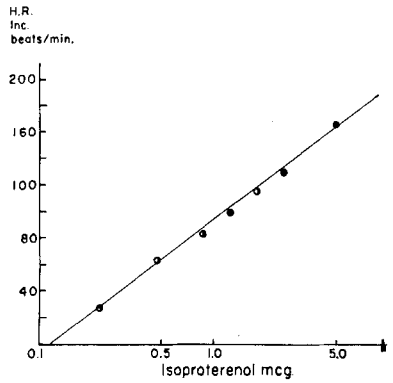


Fig. 4.—Calculated isoproterenol quantities *vs.* steady-state increases for 30-min. infusions (◑) compared with isoproterenol injected *vs.* extrapolated rate increase for rapid intravenous injection (●).

TABLE II.—CHANGE IN HEART RATES OF UNANESTHETIZED DOGS FOLLOWING ORAL ADMINISTRATION OF ISOPROTERENOL

Dose, mg.	Experiment,[a] No.	Max. Increase, %	Approx. Time for 50% Reduction in Max. Effect, min.
1.7	8	78	40
5.0	8	86	60
15.0	9	160	100

[a] Trained laboratory dogs weighing 11–16 Kg.

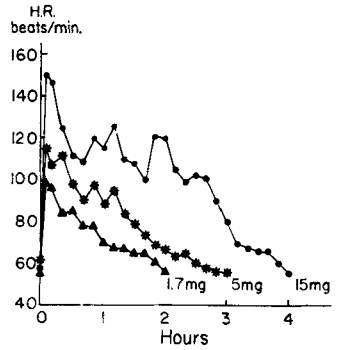


Fig. 5.—Heart rate response in dogs to the oral administration of isoproterenol in solution.

Eq. 2, where Y represents the steady-state quantity of isoproterenol.

$$Y = \frac{r}{k} \qquad \text{(Eq. 2)}$$

These data are shown graphically in Fig. 4 together with maximum heart rate increases for the rapid injection obtained by extrapolating the curves shown in Fig. 1 to $t = 0$. Agreement is remarkably close between the two sets of data and gives a log total active drug-response relationship. Parallelism exists between the dose-response (Fig. 2) and the total active drug-response relationship (Fig. 4). The per cent of isoproterenol inactivated (or otherwise lost from the active circulation) up to the time of maximum is the same for all of the doses injected. Also, the relationship shown by Fig. 4 probably is independent of the route of administration, if the activity is derived totally from isoproterenol and not a metabolite. The heart rate response is related to the amount of active drug in the circulation rather than the amount administered.

Oral Administration.—Isoproterenol administered orally in aqueous solution of 1.7–15 mg. total dose caused a prompt increase in heart rate which attained a dose related maximum within 5 min. The time from medication to one-half recovery of pulse rate was 40–100 min., and the approximate duration of any effect was 60–240 min. (Table II, Fig. 5).

Fractional doses of 1.25 and 2.5 mg./15 min. for a total dose of 15 mg. were administered and the resultant changes in pulse rate compared with those following 15 mg. given as a single medication. Results obtained are shown in Fig. 6. Relative biological efficiency of oral absorption is obtained by comparison of the areas under the curves of Figs. 5 and 6. This is shown in Table III. The total response is greater proportionately as the dose decreases, and divided doses appear to be more efficient than a single large dose.

Areas of the curve are a true reflection of absorption because of the rapid disappearance of activity after intravenous injection. When absorption is terminated, heart rate should return to the premedication level within approximately 5.5 min. (*i.e.*, within 5 half-lives). This provides a means whereby absorption can be monitored. Rates of biological

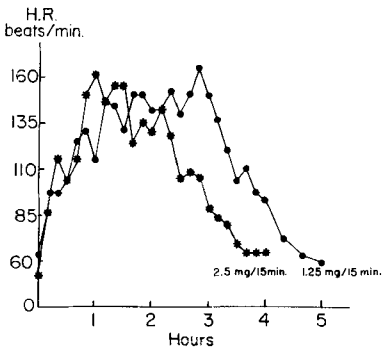


Fig. 6.—Heart rate response in dogs to the oral administration of 15 mg. isoproterenol in fractional doses.

TABLE III.—HEART RATE RESPONSE OF UNANESTHETIZED DOGS IN THE ORAL ADMINISTRATION OF ISOPROTERENOL

Isoproterenol Dose, mg.	Area, cm.²	Area (cm.²) / mg. Base	Relative Biological Activity, %[a]
1.7	10.95	6.45	206
5.0	18.20	3.64	116
15.0	46.90	3.13	100
15.0 (2.5 mg./15 min.)	58.75	3.92	125
15.0 (1.25 mg./15 min.)	80.25	5.35	171

[a] $\dfrac{\dfrac{\text{area (cm.}^2\text{)}}{\text{mg. base}} \times 100}{\left[\dfrac{\text{area (cm.}^2\text{)}}{\text{mg. base}}\right]_{15\text{-mg. dose}}}$ = % relative biological activity

availability in terms of activity can be calculated by determining the areas under the curve at various times and by expressing these as a percentage of total area. This is valid because of the small area represented by activity decay when absorption stops, *i.e.*, the half-life is small in relation to the measured duration of action.

Area comparisons between biological activity-time curves were used instead of the usual concentration-time curves. The latter are misleading in terms of biological effect because of the relationship between activity and the log of the drug concentration. Concentrations above maximum biologic response will increase the area of the concentration-time curve, and similarly, concentrations below biologic threshold will add to this area.

An estimate of the total heart rate activity after oral compared with intravenous medication is obtained by determining total area under the curves obtained from the former and expressing this as the per cent of the area under the latter. Representative results are shown in Table IV. The oral activity is less than 1.0% of the total possible activity from a 15-mg. dose of isoproterenol. This per cent activity will increase with smaller doses or with fractional doses, as previously shown.

Rates of absorption also can be calculated knowing the duration of effect for several doses. This is possible because the time required for drug elimination after absorption is completed is very small compared with the time required for complete absorption. Heart rate activity *versus* time plots (Fig. 5) are of the type expected for a compound that has a slow first-order absorption with a fast first-order elimination. For complete first-order absorption, the following equation is valid where A_0 is the dose, A is the amount of drug unabsorbed at time t, and k is the absorption rate constant. When t is chosen as the time when the effect returns to normal, then A becomes the threshold dose.

$$\log \frac{A_0}{A} = \frac{kt}{2.3} \qquad \text{(Eq. 3)}$$

A plot of log dose *versus* average duration of effect (Fig. 7) indicates that the oral threshold dose is about 0.8 mg. of isoproterenol for cardiac stimulating action in dogs. Log A_0/A can be calculated using different threshold doses (A) and plotted as a function of the average duration of effect (t).[1] Figure 8 shows that the line using a threshold dose of 0.8 mg. passes through the origin as required by Eq. 3. Experimentally, this threshold dose of 0.8 mg. was approximated. The slope of this line times 2.3 is equal to the apparent oral absorption rate constant. This is 0.735 hr.$^{-1}$ (half-life equal to 57 min.).

Rectal Administration.—Isoproterenol, in aqueous solution, was instilled into the rectum of trained unanesthetized dogs and the effect on heart rate followed, as described under *Oral Administration*. A small transient increase in heart rate was observed at a dose of 0.5 mg. Sustained responses were obtained at doses of 2–10 mg. (Fig. 9). The total cardiac response was greater for rectal than for oral medication. Area under the curve for a rectal dose of 5.0 mg. is 2.4 times greater than for the same oral dose (*cf.* Fig. 5). This greater degree and duration of activity is suggestive of a portal circulation influence on inactivation and/or increased metabolic conversion of isoproterenol when absorption takes place above the hemorrhoidal plexus.

The first-order absorption rate from the rectum was calculated in the same manner as described in the previous section for oral absorption. As indicated by Fig. 10, the rectal threshold dose is about

TABLE IV.—COMPARISON OF THE HEART RATE ACTIVITY IN DOGS AFTER ORAL AND INTRAVENOUS MEDICATION WITH ISOPROTERENOL

Isoproterenol, mcg. i.v.	Area (cm.²)	Area (cm.²)/mg.	Oral Activity, %[a]
1.25	0.478	382	0.82
2.50	0.932	370	0.85
5.00	1.560	316	0.99

[a] (3.13 × 100)/[area (cm.²)/mg.], where 3.13 = area/mg. for 15 mg. orally.

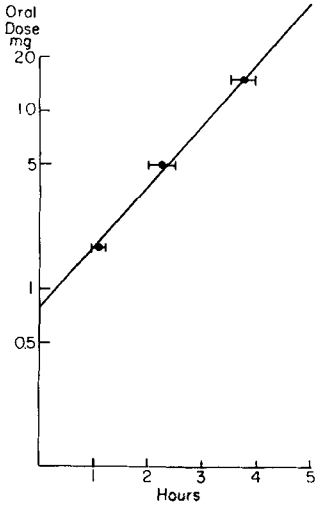


Fig. 7.—Oral isoproterenol dose *vs.* the average time for heart rate response to return to premedication value. Range of time values, 0 to +10 beats/min., for each dose are shown.

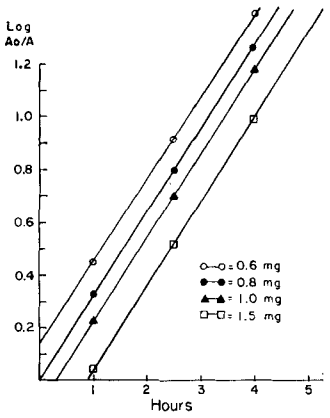


Fig. 8.—Log A_0/A *vs.* average duration of heart rate response after oral doses of 1.7, 5.0, and 15.0 mg. Points shown were calculated, assuming threshold values of 0.6, 0.8, 1.0, and 1.5 mg.

[1] Other methods which were used to determine the absorption rate constant gave the same results. Dr. G. Levy suggested the method used in this paper.

0.9 mg., and the apparent first-order absorption rate constant is 0.40 hr.$^{-1}$ (half-life equal to 104 min.).

DISCUSSION

Epinephrine disappearance from the circulation after intravenous injection or infusion to dogs resembles closely the results with isoproterenol (6, 7, 10, 11). Examination of these published data indicates a disappearance half-life of 0.75–4.0 min. Jones and Blake (7) reported this disappearance is a first-order reaction type and that epinephrine is stable in plasma and whole blood. Vendsalu (2) infused epinephrine in normal humans and followed the disappearance rate after infusion. The half-disappearance time was 2.3 min. and, according to this investigator, was a simple first-order process. Levy (12) recently has shown that pharmacologic activity usually disappears as a zero-order function of time. However, in the present case, the heart rate decay after isoproterenol is exponential probably as a consequence of pronounced nonequilibrium conditions, the half-life being in the order of a single circulation time.

The similarity of the response to isoproterenol and the disappearance of epinephrine after intravenous administration strongly suggest that similar mechanisms are involved in the handling of both amines. It may be that mechanisms outlined by Axelrod (13) and Goodall and Rosen (14) for epinephrine and norepinephrine apply also to isoproterenol, *i.e.*, rapid dilution by redistribution into tissues, *O*-methylation by catechol-*O*-methyl transferase, followed by ultimate metabolism and excretion. This assumption is supported by the recent findings of Hertting (15) in the rat administered H^3-isoproterenol. He reported that most of the isoproterenol present in the tissues 10 min. after injection was already in the *O*-methylated form. No deaminated metabolites were found.

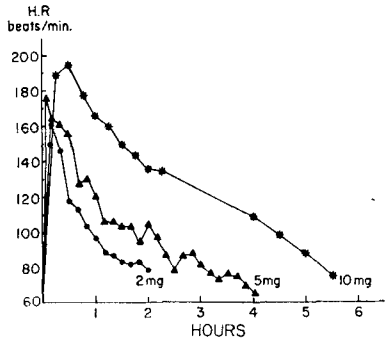


Fig. 9.—Heart rate response in dogs to the rectal administration of isoproterenol in solution.

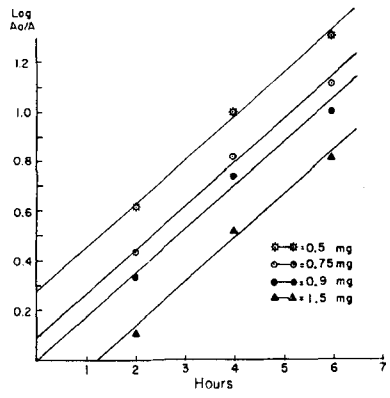


Fig. 10.—Log A_0/A *vs.* average duration of heart rate response after rectal doses of 2, 5, and 10 mg. Points shown were calculated assuming threshold values of 0.5, 0.75, 0.9, and 1.5 mg.

However, Häggendal (16) has found that conjugation rather than *O*-methylation is the predominant metabolic pathway for orally administered epinephrine in man. He suggested further that this conjugation may have been by the intestinal flora or in the intestinal mucosa since the liver cells have a large capacity to *O*-methylate catecholamines. These various processes may take place in the case of isoproterenol and contribute importantly to the observed low efficiency of venous input of biologically active drug observed in our experimentation.

Oral administration of isoproterenol provides an intestinal reservoir of drug from which diffusion into the portal circulation continues for several hours after medication. This observation is supported further by clinical experience. Sublingual medication with a rapidly disintegrating tablet of isoproterenol provides dosage that is transferred to the gastrointestinal tract soon after treatment. Dosage of 10–20 mg. provides symptomatic relief in heart block patients for 2–6 hr. (1).

The authors have found that rectal administration to dogs is more efficient than oral medication. This too is supported by clinical results. Dissatisfaction with oral medication has led to rectal use of the dosage form prepared for the former (17, 18). Kirklin (19) and Lillehei *et al.* (20, 21) have administered 5–15 mg. rectally as an oral linguet, at intervals of 2–6 hr. to patients with complete heart block, with symptomatic relief which they considered superior to oral medication. This may result from the fact that the hemorrhoidal plexus supplies blood to the systemic circulation directly as well as to the portal circulation, thereby delivering some drug directly into the inferior vena cava. The similar threshold dose of 0.8 to 0.9 mg. for oral and rectal administration indicates a greater efficiency rectally because the apparent rectal absorption rate constant is 0.54 times the apparent oral absorption rate constant.

SUMMARY

Intravenous injection or infusion of isoproterenol in dogs caused a dose-related increase in heart rate. The disappearance half-life of this effect is 0.9–1.4 min., and it appears to be a first-order process. The similarity of this physiological response to the disappearance of epinephrine from the circulation is discussed.

Estimates of isoproterenol quantities in the blood supplying the heart were calculated from infusion data, and heart rate activities were determined graphically with injection data. The agreement is shown and has given a reasonable estimate of the relationship between heart rate activity and amount of drug.

Oral administration of isoproterenol in solution to trained unanesthetized dogs caused a prompt and

dose-related tachycardia in the dosage range of 1.7–15.0 mg. total dose. Efficiency of transfer from the small intestine to the heart by way of the circulation is low, probably less than 1.0%. The total response is proportionately greater as the dose is decreased. Rectal absorption of isoproterenol in dogs is more efficient than that following oral administration.

A method of determining the first-order absorption rate constant for drugs having rapid elimination rates relative to slow absorption rates is described. Application of this method to isoproterenol indicates that the apparent oral absorption rate constant is 0.735 hr.$^{-1}$, and the apparent rectal absorption rate constant is 0.40 hr.$^{-1}$. The threshold dose both rectally and orally is quite similar (0.8–0.9 mg.). Rectally administered isoproterenol is more efficiently utilized than the orally administered drug.

REFERENCES

(1) Minatoya, H., Lands, A. M., and Portmann, G. A., *J. Pharm. Sci.*, **54**, 968(1965).
(2) Vendsalu, A., *Acta Physiol. Scand., Suppl. 173*, **1960**, 49.
(3) Cobbold, A. F., Ginsburg, J., and Paton, A., *J. Physiol. London*, **151**, 539(1960).
(4) Cookson, D. U., and Reed, C. E., *Am. Rev. Respirat. Diseases*, **88**, 636(1963).
(5) Aviado, D. M., Winuck, A. L., and DeBeer, E. T., *J. Pharmacol. Exptl. Therap.*, **122**, 406(1958).
(6) Mangan, G. F., Jr., and Mason, J. W., *Am. J. Physiol.*, **194**, 476(1958).
(7) Jones, R. T., and Blake, W. D., *ibid.*, **193**, 365(1958).
(8) Filbert, M. G., and Weller, J. M., *Proc. Soc. Exptl. Biol. Med.*, **101**, 294(1959).
(9) Teorell, T., *Arch. Intern. Pharmacodyn.*, **57**, 205, 226 (1937).
(10) Franko, B. V., Bragg, A. D., and Watts, D. T., *ibid.*, **111**, 123(1957).
(11) Pekkarinen, A., *Acta Physiol. Scand., Suppl. 54*, **1948**, 16.
(12) Levy, G., *J. Pharm. Sci.*, **53**, 342(1964).
(13) Axelrod, J., *Physiol. Rev.*, **39**, 751(1959).
(14) Goodall, M., and Rosen, L., *J. Clin. Invest.*, **42**, 1578 (1963).
(15) Hertting, G., *Biochem. Pharmacol.*, **13**, 1119(1964).
(16) Häggendal, J., *Acta Physiol. Scand.*, **59**, 255(1963).
(17) Burchell, H. B., *Circulation*, **16**, 976(1957).
(18) Robinson, S. J., *Med. Times*, **87**, 870(1959).
(19) Kirklin, J. W., *Am. J. Cardiol.*, **5**, 236(1960).
(20) Lillehei, C. W., *et al., Lancet*, **82**, 68(1962).
(21) Lillehei, C. W., *et al., J. Thorac. Cardiovas. Surg.*, **46**, 436(1963).

Enzyme Inhibitors XI

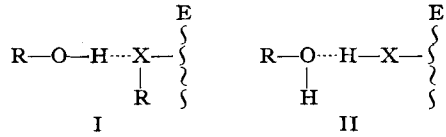
Mode of Binding of the Hydroxyl Group of Some 9-(Hydroxyalkyl)-adenines to Adenosine Deaminase

By HOWARD J. SCHAEFFER, CHARLES F. SCHWENDER, and R. N. JOHNSON

To study the mode of binding to adenosine deaminase by the hydroxyl group of 9-(2-hydroxyethyl)- and 9-(3-hydroxypropyl)adenines, a variety of 9-(2-methoxyethyl)-, 9-(3-methoxypropyl)-6-substituted purines and 9-(acetoxyalkyl)adenines were synthesized. Enzymatic evaluation of these compounds as inhibitors of adenosine deaminase revealed that they were less inhibitory than the corresponding compounds with a free hydroxyl group. These data indicate that the mode of binding of the hydroxyl group on the alkyl chain at the 9-position of adenine is by means of a hydrogen bond and that the structure of the hydrogen bond is from the hydrogen of the hydroxyl group on the inhibitor to an electronegative atom on the enzyme.

IN SEVERAL previous studies, the authors have been interested in determining which atoms and functional groups of adenosine are important for its binding to the enzyme, adenosine deaminase (1–3). In the ribofuranosyl portion of adenosine it appears that the 2′-hydroxyl group makes a contribution to binding since it has been found that in some 9-substituted adenines, those compounds with a hydroxyl group on the second or third carbon atom from the 9-position of adenine bind more tightly to the enzyme than do the corresponding nonhydroxylated compounds (4).

It is possible that the hydroxyl group of the adenine derivative exerts its influence on binding by means of a hydrogen bond.[1] One may postulate, therefore, that two different types of hydrogen bonds may be important. In the first case (I), the hydrogen of the hydroxyl group of the substrate or inhibitor forms a hydrogen bond



I II

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[1] For a discussion on the hydrogen bond, see Pimentel, J. C., and McClellan, A. L., "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960, Chap. 6.