SCIENTIFIC SECTION

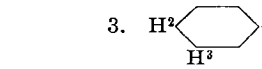
THE HYPERGLYCEMIC ACTION OF FORTY AMINES.*

BY ROBERT C. ANDERSON AND K. K. CHEN.

It is well known as a result of the work of Blum (1), Herter and Wakeman (2), Paton (3) and others that subcutaneous or intravenous injections of epinephrine are followed by a rise of blood sugar. Hyperglycemia also occurs with the homologs of epinephrine, and with tyramine and ephedrine, as shown by Morita (4), Kageyama (5), Nagel (6) and Wilson (7). It seems that pressor substances generally raise blood sugar. Nagel (6) has gone as far as to say that the elevation of the blood sugar content can be considered as a measure of the influence of a substance on the sympathetic nervous system.

In previous communications, Chen, Wu and Henriksen (8), Swanson (9) and Chen and Chen (10) reported their study on a group of amines related to ephedrine and epinephrine, mostly synthetic, with reference to their pressor action, toxicity, effect on smooth muscle organs, and other structures. The present investigation deals with the influence of forty such amines upon the blood sugar. Particular attention is drawn to any possible correlation between the pressor and hyperglycemic actions as the chemical structure varies.

The entire list of forty compounds is found in Table I. The majority of the substances are derivatives of the following three formulas:

1. $C_6H_5.CH_2.NHR$ [I]
2. $C_6H_5.CHH^1.CHR.NR^1R^2$ [II]
3.  [III]

wherein R, R^1 and R^2 = H or alkyl group, H^2 = H, OH, alkyl or alkyloxy, and H^1 and H^3 = H or OH. Four additional compounds are derivatives of indolethylamine; three, of phenyl-piperidyl-carbinols; and one, of phenyl-pyrazolone. In all, twelve primary, twenty-five secondary, two tertiary amines and one quaternary ammonium iodide are included.

Rabbits weighing approximately 2 Kg. were used for study. Aqueous solutions of the hydrochlorides of the salts were injected in the marginal vein of one ear and samples of blood were taken from that of the other. Equimolecular doses, that is, 1 cc. of a $M/10$ solution, were given, except a few, the toxicity of which was too great. In such instances, the amount was reduced. Three animals were used to study each compound. Blood samples, besides the controls, were taken 2, 5, 10, 20, 30, 40, 50 and 60 minutes after injection, and every half hour thereafter until the sugar concentration approximately returned to normal. The blood sugar was determined by the method of Hagedorn and Jensen (11). Six compounds were also studied following subcutaneous injection. The results for each were averaged and summarized in Table I.

* Scientific Section, A. PH. A., Madison meeting, 1933.

1. COMPOUNDS OF FORMULA [I].

Only two compounds, Nos. 1 and 2, belonging to the general formula, $C_6H_5.CH_2.NHR$, were studied. No. 1, $C_6H_5.CH_2.NH_2.HCl$, produced on the average a rise of 25 mg. of sugar per 100 cc. of blood, reaching its peak in 30 minutes after injection; while No. 2, $C_6H_5.CH_2.NHCH_3.HCl$, produced a rise of 18 mg., the maximum occurring in 10 minutes. In this case the primary amine seems to have a greater and more prolonged hyperglycemic action.

2. COMPOUNDS OF FORMULA [II].

Twenty-two compounds of the type, $C_6H_5.CHH^1.CHR.NR^1R^2$, were investigated. This includes the six optical isomers of ephedrine. No. 3, β-phenyl-ethylamine HCl, produced a rise of 27 mg.; while No. 4, with the OH group on the β-C atom, produced a rise of only 14 mg. No. 3 reached its peak at 40 minutes after injection, while No. 4 attained its greatest effect at the end of an hour. It is interesting to note that No. 4 was the only compound which consistently produced

TABLE I.—COMPOUNDS EXAMINED FOR HYPERGLYCEMIC ACTION.

Compound No.	Hydrochlorides of	Results. Maximal Rise of Blood Sugar mg. per 100 cc. Blood.	Time to Reach Peak after Injection (Minutes).
1	$C_6H_5.CH_2.NH_2$	25	30
2	$C_6H_5.CH_2.NHCH_3$	18	10
3	$C_6H_5.CH_2.CH_2.NH_2$	27	40
4	$C_6H_5.CHOH.CH_2.NH_2$	14	60
5	$C_6H_5.CHOH.CH_2.NHC_4H_9$	39	30
6	$C_6H_5.CHOH.CHCH_3.NH_2$	12	30
7	$C_6H_5.CHOH.CHCH_3.NH_2$ *nor-d*-Pseudoephedrine	17	30
8	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *dl*-Ephedrine	20	40
9	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *l*-Ephedrine	13	10
10	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *d*-Ephedrine	25	40
11	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *dl*-Pseudoephedrine	14	30
12	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *d*-Pseudoephedrine	20	30
13	$C_6H_5.CHOH.CHCH_3.NHCH_3$ *l*-Pseudoephedrine	30	40
14	$C_6H_5.CHOH.CHCH_3.N(CH_3)_2$ *l*-Methyl ephedrine	11	20
15	$C_6H_5.CHOH.CHCH_4.NHC_2H_5$	17	50
16	$C_6H_5.CHOH.CHCH_3.NH(CH_2.CH_2.OH)$	22	50
17	$C_6H_5.CHOH.CHCH_3.NHC_3H_7$	21	50
18	$C_6H_5.CHOH.CHCH_3.NHCH(CH_3)_2$	18	30
19	$C_6H_5.CHOH.CHCH_3.NHC_4H_9$	54	30
20	$C_6H_5.CHOH.CHCH_3.NHC_5H_{11}$	40	10
21	$C_6H_5.CHOH.CHCH_3.NH(CH_2.C_6H_5)$	71	90
22	$C_6H_5.CHOH.CHCH_3.NH(CH_2.CH_2.C_6H_5)$	45	60
23	$C_6H_5.CHOH.CHC_2H_5.NHCH_3$	22	20
24	$C_6H_5.CHOH.CHC_3H_7.NHCH_3$	22	20
25	$p\text{-}HO.C_6H_4.CH_2.CH_2.NH_2$ Tyramine	17	30
26	$p\text{-}HO.C_6H_4.CHOH.CH_2.NHCH_3$ Sympatol	35	20
27	$p\text{-}HO.C_6H_4.CHOH.CHCH_3.NH_2$	28	20
28	$p\text{-}CH_3.C_6H_4.CHOH.CHCH_3.NH_2$	27	20
29	$p\text{-}CH_3O.C_6H_4.CHOH.CHCH_3.NH_2$	18	30
30	$3,4\text{-}(HO)_2.C_6H_3.CHOH.CH_2.NHCH_3$ *l*-Epinephrine	55	60
31	$3,4\text{-}(HO)_2.C_6H_3.CHOH.CHCH_3.NH_2$ *3,4*-Dihydroxy-*nor*-ephedrine	45	30
32	$3,4\text{-}(HO)_2.C_6H_3.CHOH.CHCH_3.NHCH_3$ *3,4*-Dihydroxy-ephedrine	37	50
33	$C_8H_9.CH_2.CH_2.NH_2$ Tryptamine	24	40
34	$C_8H_9.CH_2.CH_2.NHCH_3$ Methyl-tryptamine	29	60
35	$C_8H_6.CH_2.CH_2.N(CH_3)_2$ Dimethyl-tryptamine	35	30
36	$C_8H_6.CH_2.CH_2.N(CH_3)_3.I$ Trimethyl-tryptamine quaternary ammonium iodide	50	20
37	$C_6H_5.CHOH.CH{<}\begin{matrix}CH_2-CH_2\\ \vert\quad\quad\vert\\ NH-CH_2-CH_2\end{matrix}$ Phenyl-*2*-piperidyl carbinol	3	10
38	$C_6H_5.CHOH.CH{<}\begin{matrix}CH_2-CH_2\\ \vert\quad\quad\vert\\ CH_2-NH-CH_2\end{matrix}$ Phenyl-*3*-piperidyl carbinol	10	20
39	$C_6H_5.CHOH.CH{<}\begin{matrix}CH_2-CH_2\\ \vert\quad\quad\vert\\ CH_2-CH_2-NH\end{matrix}$ Phenyl-*4*-piperidyl carbinol	10	10
40	$C_6H_5.N{<}\begin{matrix}NH-CO\\ \vert\quad\vert\\ C.CH_2.CH_2.NH_2\\ \vert\\ C.CH_3\end{matrix}$ *2*-Phenyl-*3*-methyl-*5*-pyrazolone-*4*-ethylamine	18	30

Average of three experiments.

first a fall and then a rise of blood sugar. Five minutes after injection, a fall of 9 mg. occurred. At the end of 30 minutes the blood sugar returned to its initial level, following which a rise occurred for the next 30 minutes. No. 5, a secondary amine, related to No. 4 with a butyl group replacing one H on the N atom, gave a rise of 39 mg. This agrees with the observations on other compounds of similar structure, that is, increase in the number of C atoms attached to the N atom appears to increase the hyperglycemic action. No. 6, the *dl*-product synthesized by Hartung, and No. 7, the *d*-form isolated from Ma Huang by Smith (12), differ from No. 4 in that a CH_3 group is attached to the α-C atom. The natural product produced slightly greater rise, 17 mg. as against No. 6 which caused a rise of 12 mg. Methylation on the α-C atom apparently has little influence on the hyperglycemic action since Nos. 4, 6 and 7 are nearly the same.

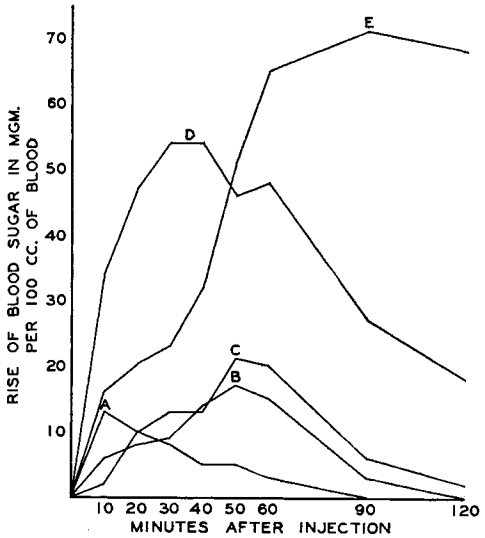


Fig. 1.—Comparison of Hyperglycemic Action of 5 Homologs.

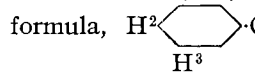
A. $C_6H_5.CHOH.CHCH_3.NHCH_3$, 1 cc. $M/10$ Solution. B. $C_6H_5.CHOH.CHCH_3.NHC_2H_5$, 1 cc. $M/10$ Solution. C. $C_6H_5.CHOH.CHCH_3.NHC_3H_7$, 1 cc. $M/10$ Solution. D. $C_6H_5.CHOH.CHCH_3.NHC_4H_9$, 1 cc. $M/10$ Solution. E. $C_6H_5.CHOH.CHCH_3.NH.CH_2.C_6H_5$, 1 cc. $M/10$ Solution.

Compounds numbered 8 to 13, inclusive, are the six optical isomers of ephedrine. Their order of activity on blood sugar is as follows: No. 9 < No. 11 < No. 12 < No. 8 < No. 10 < No. 13, the last producing a rise three times that of the first. *l*-Ephedrine, No. 9, has the least hyperglycemic action. Chen, Wu and Henriksen (8) showed that the relation in pressor action was No. 13 < No. 11 < No. 12 < No. 10 < No. 8 < No. 9, No. 9 being 35 times as strong as No. 13. Obviously, there is no correlation between the pressor and hyperglycemic actions in the optical isomers of ephedrine. No. 14, the *l*-methyl ephedrine isolated from Ma Huang by Smith (13), was found to be practically the same as *l*-ephedrine and *dl*-pseudoephedrine.

Compounds numbered 15, 17, 18, 19, 20, 21 and 22 differ from each other in the number of C atoms attached to the N atom (Table I). No. 15, the ethyl derivative, is slightly stronger than the methyl derivative (No. 9, ephedrine). The *n*-propyl derivative, No. 17, is stronger than the iso-propyl derivative, No. 18, and both in turn are stronger than the ethyl compound, No. 15. The butyl derivative, No. 19, produced a rise of 54 mg. and the amyl derivative, No. 20, showed a 40-mg. rise. However, only half the usual dose for No. 20 was given due to its high toxicity. With the addition of a benzene ring on the N atom, a further rise was observed: No. 21 caused a rise of 71 mg., and No. 22 a rise of 45 mg. Half the dosage was also used for No. 22. It appears that the hyperglycemic action increases as the number of C atoms attached to the N atom increases, as well illustrated in Fig. 1. This is contrary to

the pressor action, for Chen, Wu and Henriksen (8) showed that the pressor action decreases as the side chain on the N atom lengthens. However, when the number of C atoms linked with the α-C atom is increased, no augmentation of the hyperglycemic action occurs since Nos. 23 and 24 produced the same effect.

3. COMPOUNDS OF FORMULA [III].

Compounds numbered 25, 26, 27, 28, 29, 30, 31 and 32 are all derivatives of the general formula,  . The first five possess an OH, CH_3 or CH_3O group in the para position, while the remaining three have OH groups in both the para and meta positions. It is difficult to say what influence a single phenolic OH exerts in a compound, for No. 25 is weaker than No. 3, but in contrast to this, No. 27 is stronger than No. 6 (Table I). The introduction of a methyl radicle to the para position seems to increase the hyperglycemic activity while a methoxy group seems to decrease it. However, only one example of each was investigated.

Owing to the fact that epinephrine is a potent substance, its dose was reduced to 0.1 cc. of a 1:1000 solution which represents approximately $^1/_{200}$ of the average amount of the preceding compounds administered. Epinephrine injected intravenously produced a rise of 55 mg. per 100 cc. Compounds numbered 31 and 32 resemble epinephrine in that they both have two OH groups in the para and meta positions. Like epinephrine, they have a high pressor action (10). With No. 31, a maximal rise of 45 mg. of blood sugar was observed following the intravenous injection of 1 cc. of 1:1000 solution. No. 32 in the dosage of 1 cc. of a 1:500 solution caused a rise of 37 mg., reaching its peak in 50 minutes. It is here that the rise of blood sugar qualitatively follows the pressor action.

TABLE II.—COMPOUNDS STUDIED BY SUBCUTANEOUS INJECTION.

Compound.	Approximate Dose. Mg. per Kg.	Rise in Blood Sugar. Intravenous.	Subcutaneous.
l-Ephedrine	20	13	0
Sympatol	20	35	0
Trimethyl-tryptamine quaternary ammonium iodide	33	50	0
l-Epinephrine	0.1	55	55
3,4-Dihydroxy-*nor*-ephedrine	1.0	45	32
3,4-Dihydroxy-ephedrine	2.0	37	90

4. ADDITIONAL COMPOUNDS STUDIED.

Of the simpler indole derivatives, the order of activity on blood sugar is dimethyl-tryptamine > methyl-tryptamine > tryptamine. In pressor action, the reverse is true (10). Trimethyl-tryptamine quaternary ammonium iodide, No. 36, was given in doses of 1 cc. of $M/40$ solution. An average rise of 50 mg. was noted. The pressor action in this case is also greater than other tryptamines (10). The

three phenyl-piperidyl carbinols and the pyrazolone, No. 40, all caused a slight elevation of blood sugar.

5. SIX COMPOUNDS STUDIED SUBCUTANEOUSLY.

Compounds numbered 9, 26, 30, 31, 32 and 36 were also studied following the subcutaneous injection. The dosage employed was the same as that used for intravenous administration. As shown in Table II, ephedrine, sympatol and trimethyl-tryptamine quaternary ammonium iodide failed to show any hyperglycemic action upon the subcutaneous injection. With epinephrine and its two homologs (Nos. 31 and 32), however, a distinct rise of blood sugar was observed. There was practically no difference between the intravenous and subcutaneous injections of epinephrine. *3,4*-Dihydroxy-*nor*-ephedrine is less effective by subcutaneous injection, but *3,4*-dihydroxy-ephedrine, on the other hand, is more than twice as active as by intravenous administration.

DISCUSSION.

There are few generalizations that can be made concerning the relationship between the hyperglycemic action and chemical structure. Two primary amines are stronger than two corresponding secondary amines, and two vice versa. Methylation of a compound may therefore increase or decrease the power to raise blood sugar. The lengthening of the side chain attached to the N atom is accompanied by an increase of the hyperglycemic action. The introduction of an OH group at the para position may result in an augmentation or reduction of the sugar raising property. When a compound has a structure closely similar to that of epinephrine, such as *3,4*-dihydroxy-ephedrine and *-nor*-ephedrines, the activity is at once increased. Furthermore, they become effective in influencing the blood sugar by either intravenous or subcutaneous injections.

It is evident that there is little correlation between the hyperglycemic and pressor actions; in fact, they are often diametrically opposite. For example, the pressor action diminishes and finally disappears as the length of the side chain of the N atom increases, while the reverse is true regarding the hyperglycemic effect. The order of the pressor activity of the six optical isomers of ephedrine has been found to be *l-* > *dl-* > *d-* > *d-ψ* > *dl-ψ* > *l-ψ*. On the other hand, their action on the blood sugar is almost in reverse ratio *l-ψ* > *d-* > *d-ψ* or *dl-* > *dl-ψ* > *l-*. The rise of blood sugar becomes highest when the structure of the compound approaches that of epinephrine. Even in this case the resemblance is merely qualitative. It may be interesting to point out here that only epinephrine and its close homologs raise blood sugar by subcutaneous injection.

SUMMARY.

A series of forty compounds has been studied for their hyperglycemic action. Most of them are derivatives of one of three formulas:

1. $C_6H_5.CH_2.NHR$
2. $C_6H_5.CHH^1.CHR.NR^1R^2$
3. 

wherein R, R^1 and R^2 = H or alkyl group, H^2 = H, OH, alkyl or alkyloxy, and H^1 and H^3 = H or OH. Four derivatives of indolethylamine, three those of phenyl-piperidyl-carbinols, and one that of phenyl-pyrazolone complete the list of substances investigated.

With an increase in the number of C atoms in R, R^1 and R^2, the hyperglycemic action increases.

There is little correlation between the pressor action and hyperglycemic action as the chemical structure varies. They are often diametrically opposite.

When the structure of a compound approaches that of epinephrine, a small amount will be necessary to cause a distinct response of hyperglycemia. The epinephrine homologs also raise blood sugar by subcutaneous injection.

REFERENCES.

(1) Blum, *Arch. ges. Physiol.*, 90 (1901), 617.
(2) Herter and Wakeman, *Arch. path. Anat.*, 169 (1902), 479.
(3) Paton, *J. Physiol.*, 29 (1903), 286.
(4) Morita, *Arch. exptl. Path. Pharmakol.*, 78 (1915), 245.
(5) Kageyama, *Acta Schol. Med.*, 1 (1916), 215 and 229.
(6) Nagel, *Arch. exptl. Path. Pharmakol.*, 110 (1925), 129.
(7) Wilson, *J. Pharmacol.*, 30 (1927), 209.
(8) Chen, Wu and Henriksen, *Ibid.*, 36 (1929), 363.
(9) Swanson, JOUR. A. PH. A., 21 (1932), 1125 (No. 11).
(10) Chen and Chen, *Ibid.*, 22 (1933), 813.
(11) Hagedorn and Jensen, *Biochem. Z.*, 46 (1923), 135.
(12) Smith, *J. Chem. Soc.*, 131 (1928), 51.
(13) Smith, *Ibid.*, 130 (1927), 2056.

LILLY RESEARCH LABORATORIES, INDIANAPOLIS.

PANCREATIN AND ITS ASSAY.*

BY F. E. WILLSON.

The U. S. P. X has definite standards for the trypsin and amylase content of pancreatin. The B. P. 1932, also, has definite standards for these two enzymes in pancreatin and in addition has a standard for lipase content. The methods of determining tryptic and amylase content differ materially in the two pharmacopœias and also the standards set do not compare very closely. For this reason it is interesting to compare the different methods and observe some of the difficulties met with.

The U. S. P. Trypsin Method.—According to the U. S. P. X trypsin test, pancreatin should convert not less than twenty-five times its weight of casein into soluble proteoses. Therefore in this particular assay casein is used as the substrate. The actual method employed is essentially that known as the Fuld-Gross (1), (2). A 0.2% solution of casein is prepared by the use of sodium hydroxide. A definite quantity of a solution of the pancreatin to be tested is added to a definite quantity of the casein solution. Digestion is allowed to proceed for one hour's time, after

* Scientific Section, A. PH. A., Madison meeting, 1933.