

THE ABSENCE OF ADRENALINE-INDUCED HYPERGLYCEMIA IN PERTUSSIS-SENSITIZED MICE AND ITS RELATION TO HISTAMINE AND SEROTONIN HYPERSENSITIVITY

Charles W. Fishel, Ph.D., and Andor Szentivanyi, M.D., Denver, Colo.

THE injection of mice and rats of certain strains with killed *Bordetella pertussis* organisms alters the normal response of such animals in a number of ways.¹ Among these altered responses, the increased sensitivity of these animals to histamine and serotonin has been of major interest to our laboratory.

In the initial investigations, the grossly normal degradation of radioactive histamine and serotonin indicated that the handling of these amines by the body as a whole is unaltered in the highly sensitive pertussis-vaccinated mouse. For this reason, it was concluded that the hypersensitivity is the result of a localized hyperreactivity of the autonomic effector cells to normally nontoxic concentrations of histamine and serotonin. Attempts to modify the sensitivity with a variety of autonomic drugs produced a pattern of responses which suggest that the pertussis-sensitized mouse behaves as though it were β -adrenergically blocked.² Indeed, subsequent studies demonstrated that in the pertussis-sensitized mouse the absence of hyperglycemia following the administration of various biogenic amines results from a failure of adrenaline action.³

The present paper describes experiments showing that this failure of responsiveness to adrenaline is probably due to a blockade or some other malfunctioning of the adrenergic receptors normally associated with the inhibition of peripheral glucose uptake. It is also indicated that this glycemic abnormality is likely to be operative in the pertussis-induced hypersensitivity to histamine and serotonin.

MATERIALS AND METHODS

Mice.—Female CFW mice, weighing between 18 and 20 grams at the time of injection and obtained from a single commercial breeder, were used throughout these studies. Groups of 40 to 50 animals were housed in single plastic cages and maintained on pellets and water ad libitum.

Pertussis Vaccine.—A suspension of killed pertussis organisms, Lot No. 764645, was kindly supplied by Eli Lilly and Company. This suspension was

From the Departments of Microbiology and Medicine, University of Colorado Medical School, Denver, Colo.

This investigation was supported by grants from the National Science Foundation (G-14268) and the National Institutes of Health (AI-04191).

Received for publication May 15, 1963.

diluted 1:2 in sterile, nonpyrogenic, physiological saline and injected intraperitoneally in a volume of 0.5 ml. A period of 5 days was allowed to elapse between sensitization and the experiments outlined below.

Unlabeled Materials.—Histamine and serotonin were obtained from Nutritional Biochemical Corporation in the forms of diphosphate and creatinine sulfate, respectively. The commercially available solutions of adrenaline (Adrenalin chloride, Parke, Davis & Co.), noradrenaline (Levophed bitartrate, Winthrop Laboratories), acetylcholine (Acecoline, E. Fougera & Co., Inc.), isoproterenol (Isuprel, Winthrop, Laboratories), and regular insulin (Iletin U-40, Eli Lilly & Co.) were used. Dichloroisoproterenol (β -hydroxy-N-isopropyl-3,4-dichlorophenethylamine HCl) and Dibenamine hydrochloride (No. 00870) were kindly provided by Eli Lilly and Company. A newly synthesized β -adrenergic blocking agent, Nethalide (1,[2-naphthyl]-2-isopropylaminoethanol HCl), has been described,⁴ and it was obtained through the courtesy of Dr. J. W. Black of Imperial Chemical Industries, Ltd.

The drugs were dissolved and/or diluted to the appropriate concentration, immediately before each experiment, with physiologic, sterile, nonpyrogenic saline.

Throughout this paper, the doses of histamine are expressed in terms of free base, and those of the other amines, as well as the adrenergic blocking agents, as salts.

Radioactive Material.—D-Glucose-2-C¹⁴ containing 1.3 millicuries per millimole was obtained from Volk Radiochemical Company. The stock preparations, usually containing a total of 0.5 mc., were dissolved in 10 ml. of sterile, nonpyrogenic, physiological saline and dispensed in aliquots of 0.05 mc. with storage at -20° C. A single ampule was thawed immediately before the experiment and used for injecting 0.01 mc. into each animal.

Blood and Tissue Analyses.—Blood was obtained under light ether anesthesia and glucose concentrations determined on the deproteinized samples according to the method of Nelson.⁵ Optical densities were read in a photoelectric colorimeter (Bausch and Lomb, Spectronic 20) at 500 m μ .

Following death of the animal by exsanguination, the tissues were removed (always in the same chronological order) and the glycogen concentrations were determined by the method of Good and his co-workers,⁶ with final colorimetric glucose assays performed as above. Aliquots of the final, neutralized, glycogen hydrolysates were used for subsequent chromatographic and radioassays.

Chromatographic Analyses.—One-dimensional paper chromatography was employed to determine the relative distribution of radioactivity in the glycogen hydrolysates of the tissue samples. The solvent system consisted of a 4:1 mixture of isopropanol and water.⁷ The samples were applied to Whatman No. 1 paper and developed overnight in a saturated atmosphere. The papers were dried and the migration path of each sample was fed into a chromatogram scanner (Vanguard, Autoscaner 880) in the form of separated individual strips. All of the radioactivity was recovered as a single peak which corresponded in R_f value to that of the standard C¹⁴-glucose.

Radioassay.—Oxidized copper planchets with an area of approximately 10 sq. cm. were used for mounting the radioactive samples. Radioassays were made in duplicate in a gas flow Geiger counter operated in conjunction with an automatic sample changer and a predetermined count scaler.

The gross count rates were corrected for background and self-absorption. A minimum predetermined count of 1,280 was used for all samples.

“Relative Specific Activity” of Tissue Glycogen.—Following tissue glycogen determinations, aliquots of the same volume from the same sample were plated for radioassay. Thus, throughout this paper, the term “relative specific activity” of tissue glycogen refers to the figure obtained by dividing the net counts per minute by the tissue glycogen concentration of the same sample.

Induction of Diabetes.—The method described by Waishren⁸ was followed for the production of alloxan diabetes. Essentially, this consisted of the intravenous administration of 100 mg. per kilogram of alloxan monohydrate (Eastman Kodak Co.), which produced a marked hyperglycemia during the experimental periods and yet was not toxic for the animals.

Glucose Tolerance Test.—The test consisted of determinations of blood glucose concentrations at various intervals following the intravenous administration of dextrose (Baker). Normal, pertussis-sensitized (5 days following sensitization), dichloroisoproterenol (DCI)- and Dibenamine-injected mice were used in these experiments.

Calculations.—Means and the standard errors were calculated by standard procedures. The significance of differences between means was estimated by Student's “t” test. In the toxicity studies, the LD₅₀'s were determined according to the moving average method of Weil.⁹

RESULTS

1. *The Effect of Nethalide on the Histamine and Serotonin Resistance and on the Adrenaline-Induced Hyperglycemia of Normal Mice.*—Groups of 10 nonfasted normal mice were injected intraperitoneally with either 20 or 30 mg. of Nethalide per kilogram. (In preliminary experiments, these doses did not produce any apparent toxic manifestation in the strain employed.) Twenty minutes later the animals were given an intraperitoneal injection of either histamine or serotonin in doses indicated in Table I, and deaths were recorded over an observation period of 2 hours. Because of the extensive experience with this strain of mice in our laboratory and its known level of susceptibility² to the two amines, control groups were employed only for the higher dose levels.

According to the mortality ratios shown in Table I, the administration of 20 mg. of Nethalide per kilogram caused a marked increase in sensitivity to histamine and, to a lesser degree, to serotonin. An increase in the dose of Nethalide to 30 mg. per kilogram was followed by a further increase in the sensitivity of the animals to serotonin. Although the limited number of dose levels used in these studies does not permit a broad characterization of the dose-response relationship obtained, some suggestion of a bimodal dose-response, both to histamine and serotonin, seems to be apparent. Thus, within certain ranges

TABLE I. THE EFFECT OF NETHALIDE ON THE HISTAMINE AND SEROTONIN RESISTANCE OF NORMAL MICE

DOSE OF AMINE (MG./KG.)	HISTAMINE		SEROTONIN		
	CONTROL	NETHALIDE (20 MG./KG.)	CONTROL	NETHALIDE (20 MG./KG.)	NETHALIDE (30 MG./KG.)
500	0/10*	—	0/10	6/10	10/10
250	0/10	—	0/10	2/10	7/10
125	0/10	6/10	0/10	3/10	8/10
63	—	10/10	—	2/10	3/10
31	—	7/10	—	1/10	1/10
16	—	9/10	—	0/10	—
8	—	4/10	—	0/10	0/10

*Dead/injected.

of amine concentrations, the animals were more susceptible to lower doses than to greater amounts of the amine.

In the second experiment, the effect of Nethalide on the adrenaline-induced hyperglycemia was tested in 4 groups of 10 mice, using a different experimental arrangement in each group, as shown in Table II. In those groups in which both Nethalide (20 mg./Kg. i.p.) and adrenaline (0.3 mg./Kg. i.p.) were given, a 20 minute interval was allowed to elapse between their administrations; blood samples were taken for glucose determination 30 minutes after the injection of the second substance (i.e., adrenaline). The length of this latter time interval, as well as that of the fasting period preceding the tests, was the same in all groups. Table II shows that, while Nethalide itself does not alter the fasting blood glucose level of normal mice, it is capable of preventing the development of adrenaline-induced hyperglycemia.

TABLE II. THE EFFECT OF NETHALIDE ON THE ADRENALINE-INDUCED HYPERGLYCEMIC RESPONSE OF NORMAL MICE*

NETHALIDE (20 MG./KG.)	ADRENALINE (0.30 MG./KG.)	BLOOD GLUCOSE (MG. % $\pm$ S.E.)
—	—	114 $\pm$ 6.27
—	+	196 $\pm$ 4.81
+	—	121 $\pm$ 8.68
+	+	118 $\pm$ 7.48

*Ten animals per group.

2. *The Inverse Glycemic Effects of Small and Large Doses of Various Biologically Active Amines in Normal and Pertussis-Sensitized Mice.*—A total of 251 normal mice were fasted for 5 hours. Groups of these animals were injected intraperitoneally with progressively increasing doses of various biologically active amines listed in Table III. Thirty minutes later, blood was taken from each individual member of the group for glucose determination. The same procedure was repeated with a total of 244 mice 5 days following pertussis sensitization, except that the maximum doses of amines used in these groups were necessarily limited by the exquisite sensitivity of these animals (Table IV).

Even some of the tabulated doses employed in the pertussis groups were too large to be safely injected because they killed the sensitized mice very rapidly, and it was difficult to withdraw blood before death in amounts sufficient for analysis.

The results of Table III show that, with the exception of isoproterenol, the lower doses of all the test substances caused a marked hyperglycemia in normal mice. Isoproterenol failed to elicit this response at any of the dose levels used. As the doses of the other amines were increased, not only did larger amounts fail to increase the blood glucose concentration, but a reciprocal relationship was found to develop between the administered dose of the amine and the degree of hyperglycemia produced. Finally, beyond a certain dose level, a complete inversion of the physiologic response, i.e., a definite tendency toward hypoglycemia, was seen. The maximum doses of histamine and serotonin employed in the normal groups were still below the toxic level, whereas those of adrenaline and noradrenaline caused 42 and 85 per cent lethality, respectively.

TABLE III. BLOOD GLUCOSE LEVELS OF NORMAL MICE FOLLOWING VARIOUS DOSES OF BIOGENIC AMINES

AMINE	DOSE (MG./KG.)	LETHALITY (%)	BLOOD GLUCOSE (MG. % $\pm$ S.E.)	NO. OF ANIMALS
Adrenaline	5.0	85*	78 $\pm$ 16.70	12
	2.5	17	120 $\pm$ 26.30	10
	1.25	—	156 $\pm$ 11.37	10
	0.63	—	205 $\pm$ 8.42	19
	0.3	—	202 $\pm$ 9.51	10
	0.2	—	192 $\pm$ 10.93	10
	0.1	—	188 $\pm$ 6.54	10
Noradrenaline	5.0	42	64 $\pm$ 9.90	12
	2.5	25	75 $\pm$ 10.95	12
	1.25	—	185 $\pm$ 13.08	10
Isoproterenol	2.0	—	75 $\pm$ 12.41	10
	1.0	—	101 $\pm$ 6.71	10
	0.5	—	96 $\pm$ 8.60	10
Histamine	400.0	—	114 $\pm$ 15.09	11
	40.0	—	172 $\pm$ 8.88	11
	10.0	—	169 $\pm$ 7.07	11
	5.0	—	181 $\pm$ 8.12	11
Serotonin	400.0	—	91 $\pm$ 7.80	10
	40.0	—	83 $\pm$ 9.22	10
	20.0	—	161 $\pm$ 6.70	10
	10.0	—	188 $\pm$ 6.16	10
Saline			102 $\pm$ 3.87	22

*Twenty to 25 mice tested at each dose level.

From a comparison of the data of Tables III and IV, it seems that the glycemic responses of the normal animals at a high dose level of the various amines were closely similar to those of their pertussis-sensitized counterparts at a low dose level. In contrast to the normals, however, none of the amines at any of the dose levels used produced hyperglycemia in the pertussis-sensitized animals. Furthermore, in addition to the expected reduction in resistance of the pertussis-sensitized animals to histamine and serotonin, their resistance to

adrenaline was also lowered. For example, a dose of 1.25 mg. per kilogram was well tolerated by the normal (Table III) but was lethal for 7 per cent of the pertussis-sensitized animals, and 2.5 mg. per kilogram was lethal for 17 and 40 per cent, respectively. There was, however, no evidence of increased susceptibility to nonadrenaline or isoproterenol.

TABLE IV. BLOOD GLUCOSE LEVELS OF PERTUSSIS-SENSITIZED MICE FOLLOWING VARIOUS DOSES OF BIOGENIC AMINES

AMINE	DOSE (MG./KG.)	LETHALITY (%)	BLOOD GLUCOSE (MG. % $\pm$ S.E.)	NO. OF ANIMALS
Adrenaline	2.5	40*	60 $\pm$ 6.8	15
	1.25	7	65 $\pm$ 4.9	15
	0.625	—	107 $\pm$ 3.16	26
	0.30	—	101 $\pm$ 5.81	15
	0.20	—	98 $\pm$ 4.85	12
	0.10	—	101 $\pm$ 5.12	12
	0.05	—	96 $\pm$ 3.75	12
	0.01	—	98 $\pm$ 6.21	15
Noradrenaline	2.5	25	53 $\pm$ 3.7	9
	1.25	—	50 $\pm$ 3.0	12
	0.625	—	89 $\pm$ 6.04	9
	0.30	—	92 $\pm$ 5.21	14
Isoproterenol	2.0	—	58 $\pm$ 5.81	12
	1.0	—	67 $\pm$ 4.6	12
	0.5	—	76 $\pm$ 3.67	14
Histamine	10.0	33	73 $\pm$ 4.41	15
	8.0	29	74 $\pm$ 3.78	15
	5.0	—	99 $\pm$ 7.14	10
Serotonin	30.0	25	62 $\pm$ 8.6	12
	10.0	—	79 $\pm$ 4.0	12
	5.0	—	97 $\pm$ 5.29	12
Saline			102 $\pm$ 3.87	22

*Twenty to 25 mice tested at each dose level.

3. *Glucose Tolerance of Normal, Pertussis-Sensitized, β - and α -Adrenergically Blocked Mice.*—Four major groups of mice were employed in these experiments and were tested following a fasting period of 5 hours. The first major group consisted of normal mice that received 500 mg. of dextrose per kilogram dissolved in saline and injected intravenously in a total volume of 0.1 ml. per 10 grams of weight. At subsequent intervals, groups of 10 mice were bled and blood glucose concentrations were determined in individual members of the group. The second major group contained animals 5 days after pertussis sensitization, whereas the third and fourth groups received 10 mg. DCI per kilogram and 100 mg. Dibenamine per kilogram intraperitoneally 20 minutes prior to the glucose administration. These groups were tested in the same manner as the first group.

In Fig. 1 the means and standard errors of the changes in blood glucose concentration relative to base line are plotted as a continuous line on the ordinate against time on the abscissa. The mean values of the resting blood glucose concentrations obtained following a 5 hour fasting period in 10 normal, pertussis-sensitized, DCI- and Dibenamine-injected mice served as base line. These values were 110 ± 5.85 , 96 ± 3.60 , 114 ± 9.09 , and 129 ± 5.97 mg. per cent, respectively.

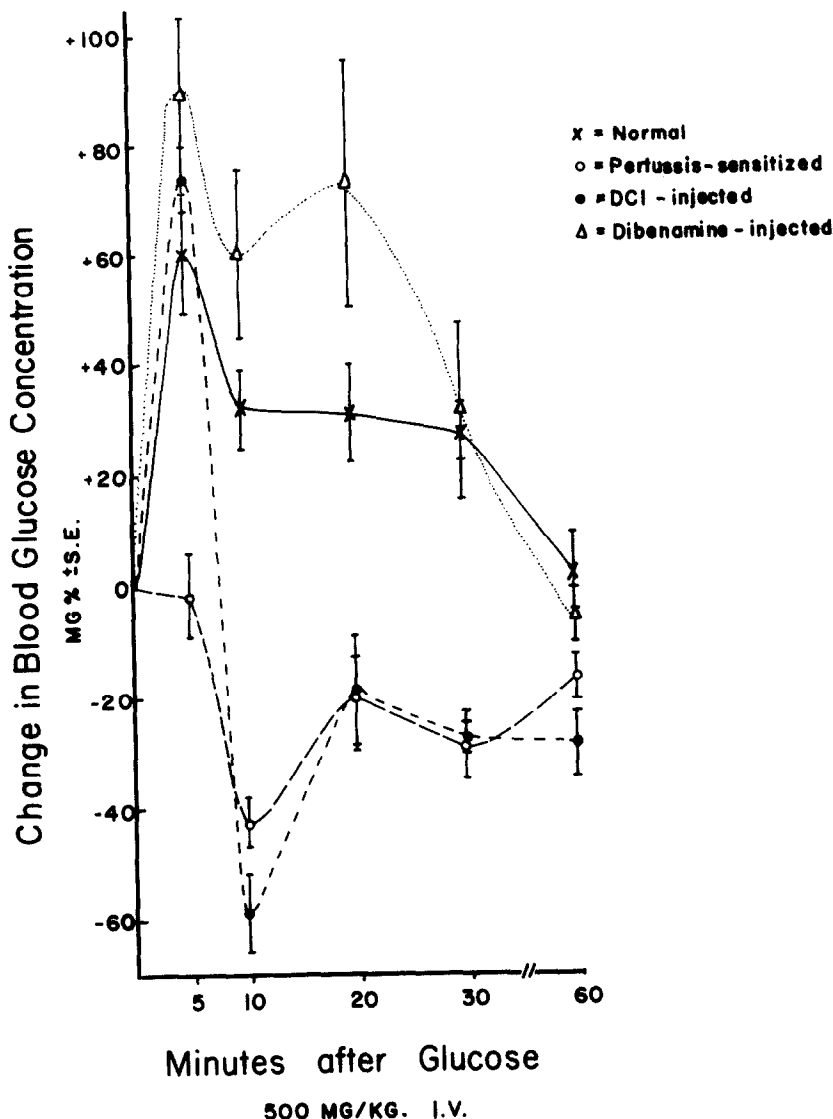


Fig. 1.—Glucose tolerance of normal, pertussis-sensitized, β - and α -adrenergically blocked mice.

It is seen that 5 minutes after the administration of glucose the normal mice showed a marked hyperglycemia, the mean value reaching 160 mg. per cent. This initial rise was followed by a lower and rather constant glucose level which, however, still was and remained significantly above the base line for the next 25 minutes. In the last 30 minutes the blood glucose concentration returned to the base line level.

In a striking contrast to the normals, the blood sugar level in the pertussis-sensitized animals at the 5 minute interval was slightly lower than the original base line. In addition, this inverted response became considerably magnified

in the next 5 minutes, exhibiting a marked hypoglycemia with a mean value of 51 mg. per cent and with little evidence of subsequent glyceimic recovery.

The blood glucose concentrations of the DCI-injected mice (β -adrenergically blocked) underwent a short-lived initial rise and a subsequent sharp decline; the blood sugar fell from 184 mg. per cent to 51 mg. per cent within a 5 minute interval. Furthermore, just as in the case of the pertussis-sensitized group, this hypoglycemic rebound was followed by only a partial restoration of the blood sugar level within the observation period. It is possible that the pertussis-injected mice exhibited a short initial rise which was missed at the time intervals used.

The glucose tolerance curve of the α -adrenergically blocked (Dibenamine-injected) mice closely resembled that of the normal animals. Following an initial, marked hyperglycemia which lasted over a period of approximately 20 minutes, the blood glucose concentration gradually returned to the base line level.

4. *Incorporation of C^{14} -Labeled Glucose Into the Tissue-Glycogen of Normal and Pertussis-Sensitized Mice.*—Following a fasting period of 5 hours, groups of normal and pertussis-sensitized mice (5 days after sensitization) were injected intraperitoneally with either 0.3 mg. of adrenaline per kilogram or an equivalent volume of saline. Twenty minutes later, each individual member of all experimental groups was given 0.01 mc. of C^{14} -glucose intravenously and, after an additional 10 minute interval, was killed by exsanguination under ether anesthesia. Samples of 8 representative tissues were removed, immediately frozen, and, with the exception of the liver, pooled for subsequent analysis.

Each value shown in Table V represents the "relative specific activity" of the glycogen obtained by pooling 8 individual samples of the respective tissue. The values given for the liver, however, represent the mean of the individually determined "relative specific activities" of 8 samples.

TABLE V. RELATIVE SPECIFIC ACTIVITIES* OF TISSUE GLYCOGEN IN NORMAL AND PERTUSSIS-SENSITIZED MICE FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 0.01 MC. D-GLUCOSE-2- C^{14}

TISSUE	NORMAL		PERTUSSIS-SENSITIZED	
	CONTROL	ADRENALINE	CONTROL	ADRENALINE
Liver	84*	54	351	—
Brain	5,170	—	7,313	2,365
Diaphragm	3,245	1,866	3,920	6,514
Heart	1,100	2,047	3,732	4,446
Kidney	7,673	1,678	7,288	4,424
Skeletal muscle	2,351	297	3,760	4,959
Spleen	2,421	474	2,391	3,600
Lung	3,670	1,036	3,389	4,697

*Counts per minute per milligram of glycogen.

Within experiment limits, the resting "relative specific activities" of kidney, spleen, and lung were closely identical in the two experimental groups, whereas there was a decidedly greater incorporation in the remaining 5 tissues of the pertussis-sensitized mice.

Following the administration of adrenaline, no comparative values are available for liver and brain, and the changes observed in heart and kidney occurred in the same direction in both groups. However, there was a striking dichotomy in the patterns of incorporation between the two groups concerning the remaining four tissues. Thus, whereas the uptake of radioactivity was uniformly and markedly decreased (two- to ninefold) in the diaphragm, skeletal muscle, spleen, and lung of the normal animals, there was an increase in the same values of the pertussis-sensitized counterparts.

5. *The Effect of Alloxan on the Blood Glucose Level of Normal and Pertussis-Sensitized Mice.*—Two major groups of mice were used in these experiments. The first was given 100 mg. of alloxan per kilogram intravenously, and one day later 73 mice were injected with pertussis vaccine, and 68 received an equivalent volume of saline, intraperitoneally. At intervals indicated in Table VI, blood was taken from representatives of each group for glucose assay. The second major group consisted of 43 mice injected with pertussis vaccine and 43 with saline. Three days later all 86 received alloxan and were bled at various intervals thereafter. The length of fasting period, the dose and route of injections, as well as the glucose assays, were the same as before.

TABLE VI. THE EFFECT OF ALLOXAN ON THE BLOOD GLUCOSE LEVEL OF NORMAL AND PERTUSSIS-SENSITIZED MICE

GROUP	TIME	BLOOD GLUCOSE			
		PERTUSSIS		SALINE	
Alloxan 1 day before pertussis or saline	2 hr.*	275 ± 10.7	(15)†	302 ± 6.6§	(15)‡
	1 da.	253 ± 23.6	(15)	380 ± 4.8	(12)
	3 da.	192 ± 25.0	(14)	388 ± 7.2	(12)
	4 da.	137 ± 24.5	(15)	407 ± 12.2	(15)
	5 da.	131 ± 14.0	(14)	399 ± 10.6	(14)
Pertussis or saline 3 days before alloxan	2 hr.†	72 ± 7.0	(14)	112 ± 11.3¶	(14)
	1 da.	84 ± 7.2	(15)	292 ± 19.8	(14)
	2 da.	137 ± 17.3	(14)	338 ± 20.5	(15)

*Interval following pertussis or saline injection.

†Interval following injection of alloxan.

‡Number of animals.

§p = 0.03.

||p < 0.001 compared to the corresponding pertussis-sensitized group.

¶p < 0.01.

The results are shown in Table VI. Regardless of whether the administration of alloxan preceded or followed the pertussis vaccination, the degree of hyperglycemia produced in the pertussis-sensitized mice was considerably lower than in their controls. It would appear that the two injections had opposite effects on the fasting glucose level, which, in the two groups receiving both injections, eventually achieved a balance slightly above the fasting glucose level of pertussis-injected mice.

6. *The Effect of Alloxan on the Histamine and Serotonin Resistance of Pertussis-Sensitized Mice.*—In these studies, the first experimental group, as in Section 5, received alloxan 3 days after pertussis vaccination, and the amine

sensitivity was tested 2 days later. The members of the second group were given alloxan one day prior to vaccination and tested 5 days later; those of the third group were vaccinated 3 days after alloxan with sensitivity assayed 5 days later. The fourth group was tested 5 days after pertussis vaccination and served as controls.

The results of Table VII show that alloxan given 3 days after pertussis had little, if any, effect on the hypersensitivity of these animals to histamine or serotonin. Conversely, when alloxan was given either one or 3 days before pertussis, the animals were found to be much more resistant to serotonin than were their non-alloxanized controls. The resistance of these groups to histamine also appeared to be increased, although to a lesser extent.

TABLE VII. THE EFFECT OF ALLOXAN ON THE HISTAMINE AND SEROTONIN RESISTANCE OF PERTUSSIS-SENSITIZED MICE

GROUP	HISTAMINE (MG./KG.)					SEROTONIN (MG./KG.)				
	500	250	125	62.5	31.3	500	250	125	62.5	31.3
Pertussis 3 days before alloxan*	8/10†	10/10	10/10	8/10	7/10	8/10	8/10	9/10	5/10	2/9
Alloxan 1 day before pertussis	—	9/10	7/10	7/10	5/10	1/10	0/8	1/10	0/10	0/10
Alloxan 3 day before pertussis	—	10/10	8/10	6/10	5/10	1/10	0/10	1/10	0/10	0/10
Pertussis control	—	—	10/10	9/9	8/10	—	—	10/10	7/9	4/10

*All groups were injected with histamine or serotonin 5 days after pertussis vaccine.

†Dead/injected.

7. *The Influence of Glucose on the Histamine and Serotonin Resistance of Pertussis-Sensitized Mice.*—Nonfasted mice, 5 days following pertussis sensitization, were used in these studies. In the first experimental group, the mice were given a total of 45 mg. dextrose in three equal doses spaced at hourly intervals. The first and last injections were given intraperitoneally, the second, subcutaneously. Thirty minutes after the last injection, groups of 10 mice were challenged with progressively increasing doses of histamine or serotonin, and deaths were recorded over a 2 hour observation period. In the second group, in place of drinking water, the mice were supplied with a 2 per cent w./v. glucose solution ad libitum over the entire preparatory period from sensitization until challenge 5 days later. Again, the animals were divided into subgroups of 10 mice before testing with histamine or serotonin. In the third group the mice received a total of 200 mg. dextrose intraperitoneally in five equally divided doses administered 30 minutes apart, and were challenged with various doses of histamine 30 minutes following the last injection. Control groups consisted of pertussis-sensitized mice which were not given glucose.

From the mortality ratios presented in Table VIII, it is seen that the serotonin sensitivity of pertussis-injected mice was strikingly reduced by glucose, regardless of whether it was administered during the preparatory period

TABLE VIII. THE INFLUENCE OF GLUCOSE ON THE HISTAMINE AND SEROTONIN RESISTANCE OF PERTUSSIS-SENSITIZED MICE

GROUP	CHALLENGE DOSE OF AMINE (MG./KG.)						
	500	250	125	63	32	16	8
Glucose I*							
Histamine	—	—	10/10§	9/10	10/10	8/10	5/10
Serotonin	5/10	2/10	1/10	0/10	0/10	—	—
Glucose II†							
Histamine	—	—	10/10	10/10	9/10	9/10	—
Serotonin	—	1/10	0/10	0/10	—	—	—
Glucose III‡							
Histamine	—	—	10/10	—	10/10	8/10	—
Control							
Histamine	—	—	—	10/10	10/10	9/10	6/10
Serotonin	—	—	10/10	10/10	8/10	2/10	0/10

*A total of 45 mg. glucose given in 3 equal doses at hourly intervals.

†Supplied with 2 per cent glucose for drinking water from time of pertussis vaccination until challenge.

‡A total of 200 mg. glucose given in five equal doses at 30 minute intervals.

§Dead/injected.

or prior to challenge. In contrast, neither of these procedures appreciably altered the hypersensitivity to histamine.

8. *The Effect of Insulin on the Resistance of Normal Mice to Histamine and Serotonin.*—Three general groups of mice were used in these studies. The first group was not fasted, whereas the second group was employed following a 5 hour fasting period. Otherwise these two groups were handled in the same manner. The animals were injected intraperitoneally with 0.4 units of insulin per kilogram, i.e., with a dose which was shown to be safely below the toxic level (Table X). Thirty minutes later, groups of 10 mice were challenged intraperitoneally with progressively increasing doses of histamine or serotonin, and deaths were recorded over a 2 hour observation period. Control groups consisted of normal mice which were not given insulin and which were challenged following a 5 hour fasting period.

The results presented in Table IX show that the doses of histamine and serotonin used in these experiments were all well tolerated by the control ani-

TABLE IX. THE EFFECT OF INSULIN ON THE RESISTANCE OF NORMAL MICE TO HISTAMINE AND SEROTONIN

GROUP	CHALLENGE DOSE OF AMINE (MG./KG.)									
	HISTAMINE					SEROTONIN				
	300	150	75	38	19	300	150	75	38	19
I. Fed	2/10†	1/10	1/10	0/9	0/9	7/10	10/10	8/10	9/10	2/10
II. Fasted*	—	10/10	9/10	8/10	16/20	—	10/10	7/10	10/10	8/10
III. Control* (no insulin)	0/10	0/10	0/10	0/10	—	0/10	0/10	0/10	—	—

*Animals fasted 5 hours prior to insulin injection; challenge 30 minutes later.

†Dead/injected.

mals. In contrast, a markedly increased sensitivity to serotonin was found in the insulin-injected, nonfasted animals. The resistance of this group to histamine was not as strikingly affected, although a slight reduction did occur. On the other hand, the same amount of insulin administered to fasted animals sensitized them to both histamine and serotonin equally well. There was also some indication of the bimodal dose-response noted previously in pertussis-sensitized and β -adrenergically blocked mice.² For instance, the fasted insulin-injected mice were more susceptible to 38 than to 300 mg. serotonin per kilogram.

9. *Insulin Resistance of Normal and Pertussis-Sensitized Mice.*—Groups of normal and 5-day pertussis-sensitized mice were challenged, following a 5 hour fasting period, with progressively increasing doses of insulin administered intraperitoneally and deaths were recorded over a 3 hour period.

Table X shows that the LD₅₀'s were found to be 1.8 and 2.6 units per kilogram for normal and pertussis-sensitized mice, respectively. Since the 95 per cent confidence intervals overlapped, this difference is not considered to be significant.

TABLE X. INSULIN RESISTANCE OF NORMAL AND PERTUSSIS-SENSITIZED MICE*

DOSE OF INSULIN (UNITS/KG.)	LETHALITY	
	NORMAL	PERTUSSIS
3.05	10/10	9/10
2.03	8/10	6/10
1.35	7/10	2/10
0.90	5/10	1/10
0.60	0/10	0/10
0.40	0/10	0/10
LD ₅₀	1.80 (1.2-2.5)†	2.5 (2.1-3.2)†

*Animals were fasted for 5 hours prior to test; injections made intraperitoneally, 10 per group.

†95 per cent confidence interval.

DISCUSSION

Previous work in our laboratory showed that the hyperglycemia following histamine and serotonin is basically an adrenaline-mediated response in the normal mouse.³ Under these conditions, the repeatedly demonstrated absence of hyperglycemia to these amines in the pertussis-sensitized mouse was interpreted to be the result of a failure of either adrenaline release or action. The evidence for the second alternative was clear since, in the pertussis-sensitized mouse, the release of medullary adrenaline by histamine or serotonin was found to be accentuated,³ and adrenaline itself (administered exogenously) did not produce hyperglycemia.

This failure of responsiveness to adrenaline could be attributed to an inverse glycemic action of excessive amounts of adrenaline, liberated endogenously by histamine or serotonin. There are two lines of evidence which seem to point in this direction. In previous experiments, the catechol-depleting efficiency of histamine and serotonin from the medullary tissue was considerably greater in pertussis-sensitized than in normal mice. For instance, the amount of histamine

required to give a comparable adrenaline-release in the two groups was approximately 100-fold less in the pertussis-sensitized than in normal mice.³ In addition, the present experiments showed that beyond a certain dose level there is an inverse relationship between the administered dose of the amine and the degree of hyperglycemia produced (Table III). Thus, on the one hand, excessive amounts of adrenaline and noradrenaline are known to be liberated in the pertussis-sensitized animal following histamine or serotonin and, on the other hand, large amounts of catechols are known to produce an inverse glycemic effect. The net result of this combined effect could be a failure of the blood sugar to rise in the pertussis-sensitized animal after the injection of histamine or serotonin.

The experiments reported in Tables III and IV were designed to test the above hypothesis. The results showed that, in the pertussis-sensitized mouse, small doses of adrenaline or of other amines are just as incapable of producing hyperglycemia as are larger amounts. These results provide, therefore, strong evidence against the hypothesis of an inverse glycemic effect.

In the pertussis-sensitized animal the failure of responsiveness to adrenaline is confined to certain areas of adrenaline action. For example, the hepatic glycogenolytic activity of adrenaline was found to be not only retained but exaggerated.³ The combination of exaggerated responses in one area of adrenaline activity with completely failing responses in another area of adrenaline activity is a strong indication of a selective adrenergic blockade.¹⁰ That, in the pertussis-sensitized animal, a selective blockade, or some other malfunctioning of the β -adrenergic receptors, may indeed be responsible for the lacking hyperglycemia is suggested by the fact that DCI (a specific β -receptor blocking agent) produced the same split pattern of adrenaline activity in the normal mouse.³ A further justification of this analogy is provided by the present observations that Nethalide, another highly specific β -blocking agent, is also capable of both preventing the hyperglycemia to adrenaline and of causing a degree of hypersensitivity to histamine and serotonin comparable to that produced by pertussis or DCI (Tables I and II).

Whatever the exact nature of this selective malfunctioning of the adrenergic receptors in the pertussis-sensitized animal, an important fact is that the enhanced hepatic glycogenolysis in the face of lacking hyperglycemia leaves us with an apparent deficit in accounting for the fate of glucose following adrenaline.

Such a phenomenon may arise from one or a combination of several of the following abnormalities: (1) a hepatic glycogen content insufficient to give an adequate rise in blood glucose following adrenaline; (2) a failure of glucose derived from hepatic glycogen breakdown to be released into the extracellular space, including the blood, and (3) an increased peripheral utilization of glucose.

The first hypothesis can be readily eliminated. In previous studies, the resting hepatic glycogen level was found to be within the normal range and glycogenolysis, following adrenaline, enhanced.³ Consequently, the metabolically available hepatic glycogen must have been quantitatively sufficient to give a degree of hyperglycemia ordinarily seen following the dose of adrenaline em-

ployed in the experiments. The same facts argue against the second hypothesis since, over the 5 day preparatory period, the presence of progressively increasing amounts of intrahepatically retained glucose would be expected to block glycogenolysis with a resultant accumulation of hepatic glycogen. Conversely, the third hypothesis is clearly supported by the greatly augmented glucose tolerance of the pertussis-sensitized animals (Fig. 1), and by the increased incorporation of radioactive glucose into the tissue glycogen of pertussis-sensitized mice under resting conditions (Table V).

When other regulatory factors are functioning properly, the degree of peripheral uptake of glucose will depend upon a momentary balance between the antagonistic activities of catecholamines and insulin which affect the utilization in opposite directions. Therefore, an abnormal increase in peripheral uptake of glucose may reflect (a) a failure of the inhibiting activity of adrenaline with a resultant relative hyperinsulinism, (b) a primary hyperinsulinism, or (c) a combination of the former two conditions.

The present experiments provide evidence for the first alternative and against the other two. Confirming the earlier observations of others,^{11, 12} the degree of hyperglycemia produced in the pertussis-sensitized mice by alloxan was considerably lower than that seen in the normal animals (Table VI). Thus, the exclusion of the sole cellular source of insulin did not eliminate the original abnormality in the peripheral utilization of glucose. Further, in the non-alloxanized pertussis-sensitized animals, adrenaline was incapable of correcting the same defect, i.e., it failed to reduce the peripheral uptake of radioactive glucose into the glycogen of skeletal muscle, diaphragm, spleen, and lung of such animals (Table V). It is also clear that the establishment of a β -adrenergic (DCI) blockade affected the glucose tolerance of normal animals in a manner similar to pertussis sensitization, whereas an α -adrenergic (Dibenamine) blockade did not alter the normal tolerance to glucose (Fig. 1).

It seems permissible to conclude, therefore, that the adrenergic receptors (probably beta) associated with the inhibition of peripheral glucose uptake appear to be blocked in the pertussis-sensitized animal which lead, in essence, to a reduction in the degree of antagonism to insulin which exists under normal circumstances. Relative hyperinsulinism occurs largely because of this effect, and consequently, hypoglycemia may follow.

Although the pertussis-sensitized animals show a normal resistance to insulin (Table X), the glycemic maladjustment to the various biogenic amines does not appear to be a trivial by-product of the postulated β -adrenergic blockade, but probably is operative in the induction of the hypersensitive state. This is indicated by the fact that both alloxan diabetes (Table VII) and long-term as well as acute administration of glucose (Table VIII) were found to restore the normal resistance of the pertussis-sensitized mouse to serotonin. Conversely, insulin was found to sensitize normal mice to histamine and serotonin (Table IX).

On the other hand, the development of the hypersensitivity cannot be accounted for in terms of the glycemic defect alone, since the absence of hyperglycemia after adrenaline is demonstrable at least as early as 2 hours following

pertussis sensitization.³ This is in contrast with the relatively late appearance of the hypersensitivity (4 to 6 days after sensitization). Furthermore, according to our most recent findings, a malfunctioning of the β -adrenergic receptors is not confined to those engaged in the normal inhibition of peripheral glucose uptake. For example, the normal increase in heart rate following administration of adrenaline does not occur in pertussis-sensitized mice. Thus, the adrenergic receptors associated with the positive chronotropic function of the heart are also affected. In terms of the dual classification theory of Ahlquist,¹³ these are believed to be β -receptors.^{14, 15} This, together with the other observations, suggests a rather widespread involvement of the β -adrenergic receptors in the pertussis-sensitized mouse.

In conclusion, it seems that the pertussis vaccine possesses a component or causes the host to elaborate a substance with a steric configuration complementary to the β -adrenergic receptors. Because of its natural affinity, this substance combines with the β -receptors and occupies the site normally available for the adrenergic transmitter, i.e., adrenaline and/or noradrenaline. When such an animal is challenged with histamine or serotonin, the antagonistic action of the excessively released catecholamines is blocked at the β -receptor level, leaving the α -adrenergic activities unopposed. The resultant imbalance produces a combination of unfavorable metabolic, and consequently smooth muscle, as well as neural adjustments, which culminates in a reduced resistance to histamine and serotonin.

SUMMARY

Experiments are described which were designed to investigate the nature of the absence of adrenaline-induced hyperglycemia in pertussis-sensitized mice and its relation to histamine and serotonin hypersensitivity.

It is shown that the absence of hyperglycemia after administration of adrenaline cannot be attributed to an inverse glyceemic action of excessive amounts of adrenaline liberated endogenously by histamine or serotonin. On the other hand, several observations do suggest that a blockade or some other malfunctioning of the adrenergic receptors (beta), normally associated with the peripheral inhibition of glucose uptake, is responsible for the absence of hyperglycemia. This is indicated by the unusually augmented glucose tolerance of pertussis-sensitized mice, by the increased incorporation of radioactive glucose into the tissue glycogen of such animals, and by the failure of adrenaline to correct this abnormality. Furthermore, the establishment of a β -adrenergic blockade (DCI) affected the glucose tolerance of normal animals in a manner similar to pertussis sensitization, whereas an α -adrenergic blockade (Dibenzamine) did not alter the normal tolerance to glucose.

Although the pertussis-sensitized animals showed a normal resistance to insulin, the abnormally increased peripheral uptake of glucose in such animals is likely to be operative in the induction of hypersensitivity to histamine and serotonin. This is indicated by the fact that both alloxan diabetes and long-term, as well as acute, administration of glucose were found to restore the normal

resistance of the pertussis-sensitized mouse to serotonin. Conversely, insulin was shown to sensitize normal mice to histamine and serotonin.

Deep appreciation is expressed to Drs. David W. Talmage and Richard Thompson for their valuable suggestions during the course of these investigations. The technical assistance of Miss Jean Baughman and Mrs. Carla Gilbert is gratefully acknowledged.

REFERENCES

1. Kind, L. S.: The Altered Reactivity of Mice After Inoculation With *Bordetella pertussis*, Bact. Rev. **22**: 173, 1958.
2. Fishel, C. W., Szentivanyi, A., and Talmage, D. W.: Sensitization and Desensitization of Mice to Histamine and Serotonin by Neurohumors, J. Immunol. **89**: 8, 1962.
3. Szentivanyi, A., Fishel, C. W., and Talmage, D. W.: Adrenaline Mediation of Histamine and Serotonin Hyperglycemia in Normal Mice, and the Absence of Adrenaline-Induced Hyperglycemia in Pertussis-Sensitized Mice, J. Infect. Dis. (In press.)
4. Black, J. W., and Stephenson, J. S.: Pharmacology of a New Beta-Receptor-Blocking Agent (Nethalide), Lancet **2**: 311, 1962.
5. Nelson, N.: A Photometric Adaptation of the Somogyi Method for the Determination of Glucose, J. Biol. Chem. **153**: 375, 1944.
6. Good, C. A., Kramer, H., and Somogyi, M.: The Determination of Glycogen, J. Biol. Chem. **180**: 485, 1933.
7. Smith, L.: Chromatographic and Electrophoretic Techniques, New York, 1960, Interscience Publishers, Inc., vol. 1, p. 248.
8. Waisbren, B. A.: Alloxan Diabetes in Mice, Proc. Soc. Exper. Biol. & Med. **67**: 154, 1948.
9. Weil, C. S.: Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions in Their Use, Biometrics **8**: 249, 1952.
10. Nickerson, M.: Blockade of the Actions of Adrenaline and Noradrenaline, Pharmacol. Rev. **11**: 443, 1959.
11. Parfentjev, I. A., and Schleyer, W. L.: The Influence of Histamine on the Blood Sugar Level of Normal and Sensitized Mice, Arch. Biochem. **20**: 341, 1949.
12. Ganley, O. H.: Studies on the Prevention of Sensitization by *B. Pertussis* in Alloxan Diabetic Mice, Canad. J. Biochem. & Physiol. **40**: 1179, 1962.
13. Ahlquist, R. P.: A Study of the Adrenotropic Receptors, Am. J. Physiol. **153**: 586, 1948.
14. Moran, N. C., and Perkins, M. E.: Adrenergic Blockade of the Mammalian Heart by a Dichloro Analogue of Isoproterenol, J. Pharm. & Exper. Therap. **124**: 223, 1958.
15. Dresel, P. E.: Blockade of Some Cardiac Actions of Adrenaline by Dichloroisoproterenol, Canad. J. Biochem. & Physiol. **38**: 375, 1960.