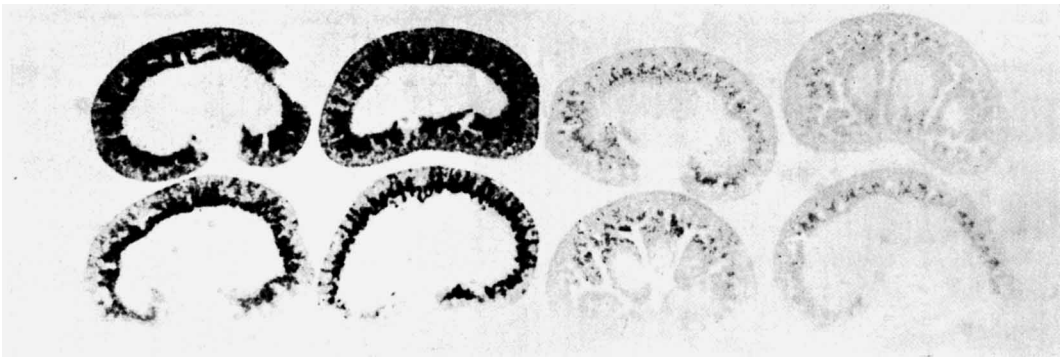

FIG. 1.
Radioautograph of kidneys, 24 hr after administration of radiomercuric chloride. Left, control group. Right, experimental (proteinuric) group. Eastman Kodak NTB plate, 10 μ emulsion, 10 million counts per cm^2, magnification about 3×.

toxicity of inorganic mercuric chloride as it has upon the mercurial diuretic.

In our previous work(3) we were unable to demonstrate that mercurial diuretic was bound by serum protein. However, since that time Milnor(6) has succeeded in such a demonstration. It is well known that mercuric ion is bound by protein, and this experiment shows that proteinuria prevents the mercuric ion from localizing in the kidney. It would seem reasonable to suppose that, in view of Milnor's work, an organic mercurial diuretic would behave in the same way.

Summary. When radioactive mercuric chloride is administered to animals rendered proteinuric by the administration of bovine albumin, less radiomercury is localized in the renal cortex than in that of the control animals. Diminished renal toxicity is associated with a diminished localization of radiomercury in the kidney.

6. Milnor, J. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 63.

Received April 11, 1951. P.S.E.B.M., 1951, v77.

Sensitivity of Mice to Histamine During Respiratory Infection by *Hemophilus pertussis.* (18682)

MARGARET PITTMAN.

From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute, Bethesda, Md.

It was first shown by Parfentjev and Goodline(1) that pertussis vaccines can markedly increase the sensitivity of mice to histamine. Pittman(2) has reported that vaccines of relatively low toxicity may show a direct correlation in ability to induce sensitization and protection in mice; a toxic vaccine, however, induced better protection than sensitization. During her study, it was observed that a higher percentage of vaccinated mice which had survived an intracerebral inoculation of *H. pertussis,* were sensitive to histamine than other mice correspondingly vaccinated but not challenged(3). This suggested that the living bacteria might also be capable of inducing a sensitivity and that this property might have some bearing on the clinical manifestations of the disease in man. The intracerebral route of infection in the mouse is highly artificial

1. Parfentjev, I. A. and Goodline, A., *J. Pharm. Exp. Therap.,* 1948, v92, 411.

2. Pittman, M., *J. Infect. Dis.,* in press.

3. Pittman, M., Abstracts, Fifth International Congress for Microbiology, Rio de Janeiro, Brazil, 1950.

TABLE I. Mortality and Time of Death in Mice Infected Intranasally.

Exp. No.	No. of bacteria inoculated, ×1000	Period of observation, day	Deaths			
			D/n	%	Avg	±S.E.
9	10000	50	27/ 80	33.75	16.1	±1.01
	1000		11/ 80	13.75	23.5	±2.06
14	10000	45	49/110	44.5	17.7	±0.85
	1000		26/ 90	29.0	19.3	±0.98
	100		11/ 50	22.0	21.1	±2.73
26	50	28	18/178	10.1	20.8	±1.02

D/n = Deaths over No. inj.

and unless vaccinated mice do not survive. By the intranasal route(4), however, a high degree of infectivity with low mortality can be induced. This infection also has the advantage that, in some respects, it resembles the human infection. In the present paper, there will be reported some observations on the histamine sensitivity and the presence of gross lesions and of *H. pertussis* in the lungs of mice inoculated intranasally with *H. pertussis* and on the resistance of recovered mice to an intracerebral inoculation of *H. pertussis*.

Materials and methods. A culture of *H. pertussis* (No. 1/92) which had a high virulence for mice by the intracerebral route, was grown for 20 hours on a Bordet-Gengou agar slant, suspended in a solution of 1.0% protolysate (a tryptic digest of casein) and 0.5% sodium chloride and then instilled in the nares of anesthetized female mice, in a volume of 0.05 ml. Only female mice were used because both normal and vaccinated female mice are more sensitive to histamine than male mice(5). At intervals during the course of the infection the sensitivity to histamine diphosphate was determined by injecting intraperitoneally 100 mg per kg in 0.2 ml distilled water. The lungs of all mice that died or had survived for 2 hours after the injection, were examined for gross lesions and cultured on Bordet-Gengou agar slants. Some experiments carried normal mice for histamine controls, these mice were not sensitive to the dose of histamine used and are not reported in the results. The LD_{50} of histamine for the normal female mouse is around 1900 mg per kg(5).

Results. Mortality and time of death. In Table I are given the times and percentages of death of the mice in relation to the inocula of culture observed in 3 experiments. In the first two, mice were withdrawn at varying intervals for histamine sensitivity determinations. In the former, withdrawals were started earlier than in the latter. This may explain the lower mortality obtained in it. Experiment 26 was observed for specific deaths for only 28 days. It may be seen that the number and time of death were related to the amount of culture injected but even with the largest dose more than 50% survived, while the average time of death for the four doses ranged from 16.1 to 23.5 days. The earliest death was on 5th day and the latest on 41st day. An infection with this duration and relatively low mortality afforded an excellent opportunity to observe the development and duration of histamine sensitization in mice infected with *H. pertussis*.

Histamine sensitization. In the experiment reported in Table II, two groups of mice of 100 each were injected with 10- and 1 million bacteria, respectively, then at intervals between 5 and 50 days, mice in groups of 10 or more were tested for sensitivity to histamine. The larger dose of culture induced, by the end of 10 days, a high degree of sensitivity, which persisted for many days; at the end of 50 days, 33% of the remaining mice were still sensitive. The LD_{50} of histamine at 15 days was 47 mg per kg. Following the inoculation of the smaller dose, the rise in sensitization was slower. From this experiment and No. 14 reported in the next section, it appears that by 45 or 50 days when the mice had started to recover from the infection that sensitization was decreasing. At the end of 5 days, there

4. Burnet, F. M. and Timmins, C., *Brit. J. Exp. Path.*, 1937, v18, 83.

5. Pittman, M., *J. Infect. Dis.*, in press.

TABLE II. Histamine Sensitization in Relation to Pathology and Infection. (Exp. 9).

No. of bacteria inoculated, ×1000	Histamine diphosphate: After culture, days	Amount, mg/kg	Deaths: D/n	Deaths: %	Gross lung pathology: P/D	P/S	Total %	*H. pertussis* recovered: C/D	C/S	Total %
10000	5	100	6/10	60	6/6	4/4	100	4/6*	3/4	70*
	10	100	10/10	100	10/10		100	10/10		100
	15†	100	5/5	100	5/5			4/5		
		50	6/10	60	6/6	4/4	100	6/6	4/4	95
		25	0/5	0		5/5			5/5	
	20	100	8/10	80	8/8	1/2	90	6/8	1/2	70
	30	100	10/13	77	9/10	2/3	85	1/10	1/3	15
	50	100	3/9	33	2/3	3/6	56	0/3	0/6	0
1000	5	100	1/10	10	1/1	6/9	70	1/1	4/9	50
	10	100	5/10	50	5/5	0/5	50	5/5	2/5	70
	15	100	7/10	70	7/7	1/3	80	6/7	1/3	70
	20	100	5/10	50	5/5	1/5	60	4/5	0/5	40
	30	100	6/16	38	4/6	1/10	32	0/6	0/10	0
	50	100	11/22	50	10/11‡	1/11	50	0/11	0/11	0

* Two cultures were overgrown with a contaminant.

† LD_{50} of histamine at 15 days = 47 mg/kg. 2 σ limits 37.7 and 58.6 mg/kg. D = deaths; S = survival; n = No. inj.; P = lung lesions; C = positive cultures.

‡ In some the lungs appeared grey, unlike those observed in other mice.

was a higher incidence of lung involvement than sensitization, thereafter the presence of gross lesions and sensitization was closely correlated. Through the first 15 days the recovery of *H. pertussis* was correlated with the pathology. At 30 days and later, bacteria were recovered from very few mice. In Table III is given a summary of observations made in 4 experiments on the incidence of lung lesions and positive *H. pertussis* cultures in relation to death from histaminic shock. Of the mice that died, both lung pathology and positive cultures were obtained from 58.8%, while 84.8% showed pathology and from 64.8% positive cultures were obtained. The incidence of each manifestation was significantly lower in the mice that survived. If it is taken into consideration that in the mice which survived, the highest incidence of lung lesions was in those tested early, and that in the mice which died the highest incidence of negative cultures was in those tested late, then we do indeed have a close correlation between histamine sensitization and the *H. pertussis* infection in the mice.

TABLE III. Correlation of Lung Pathology and Lung Cultures with Histamine Sensitization of Mice from 4 Experiments.

Lung: Pathology	Lung: Culture	Response to histamine: Died, 182 No.	Died, 182 %	Survived, 116 No.	Survived, 116 %
+		154	84.6	34	29.3
	+	118	64.8	22	19.0
—		28	15.3	82	70.7
	—	64	35.1	94	81.0
+	+	107	58.8	13	11.2
+	—	47	25.8	21	18.1
—	+	11	6.0	9	7.8
—	—	17	9.3	73	62.9

Resistance of recovered mice. In several experiments, the mice that had survived the inoculation of culture intranasally were given an intracerebral injection of a culture of another strain of *H. pertussis* (No. 18323). Most of the mice were resistant. The results of one experiment (No. 14) are given in Table IV. Following the intranasal injection, the course of the infection and sensitivity had been similar to that reported for experiment 9 in Table II. Mice from 3 groups, which had been given 10,000-, 1,000-, and 100 thousand bacteria, respectively, were tested for histamine sensitivity at 15, 30, and 45 days. The percentages of shock deaths of mice from the respective groups at 15 days were 80, 90, and 60; at 30 days, 100, 82, and 100; and at 45 days, 20, 41, and 40. A high percentage of those that died showed lung lesions. At 15 days the percentage of positive lung cultures

TABLE IV. Resistance of Mice to Intracerebral Infection After Intranasal Inoculation.

No. of bacteria inoculated			*H. pertussis* recovered from†		
			Brain		Lung
Intranasally, ×1000	Intracerebrally,* ×1000	No. survivals/No. inj. intracer.	Survivals	Dead	Dead
10000	100	12/12	1		
	20	10/12	1	1	0
	4	11/12	1	0	1
1000	100	13/15	0	2	1
	20	15/15	1		
	4	13/14	1	1	0
100	100	8/12	1	2	0
	20	6/10	0	2	0
	4	8/10	0		
	Total/n	96/112	6/96	8/12	2/12
	%	85.7	6.25	66.7	16.7
		Controls			
0	100	1/10	1		
	20	0/10			
	4	4/10	1		
	2	3/10	0		
	.4	4/10	0		
	.08	10/10	1		

* 45 days after intranasal inoculation. LD_{50} of culture = 550 bacteria.

† Cannibalism prevented the culturing of 4 mice. Cultures were not made of the lungs of mice that survived or of the brain of the control mice that died.

was also high; at 45 days, in the first group, all cultures were negative, but in the other two, 18 and 20%, respectively, were positive. This incidence is higher than was obtained in experiment 9.

At the time of the last sensitivity test the remaining mice in each group were injected intracerebrally with 3 doses of culture, respectively, as indicated in Table IV. They were observed for 14 days; those that had symptoms of brain involvement at the end of this time were counted among the dead mice. There was no apparent difference in the response to the different intracerebral doses in the mice that had been given the same intranasal inoculum. It appears, however, that better protection was obtained in the mice that had received the two larger intranasal inocula. Eighty-six per cent of all mice survived; from 6 of these, although the mice showed no symptoms, positive brain cultures were obtained.

Discussion. Mice with an induced *H. pertussis* respiratory infection developed a high degree of sensitivity to histamine which was comparable to that induced by 300- to 1000 million bacteria of good mouse-protective pertussis vaccines (non-precipitated) (2). It differed from the latter in that the maximum degree of sensitivity was not reached for 10 or 15 days, it persisted much longer and it was closely correlated with the presence of lung lesions. The development of sensitivity appears to have some relation to the multiplication of the bacteria in the lungs and not to the lesions *per se* as they were present before a high degree of sensitivity developed but sensitivity persisted longer than the bacteria. With vaccine, the peak of sensitivity is reached in 4 or 5 days, followed by a rapid drop.

The course of the infection and the histamine sensitivity in the mouse strikingly parallels the onset and duration of paroxysmal coughing in the child. In the child, there is an incubation period of 7 to 10 days, a localization of the bacteria in the respiratory tract and lungs, and a paroxysmal cough of long duration which persists after *H. pertussis* can no longer be isolated. These manifestations of pertussis set it apart from other bacterial respiratory infections and they suggest that some form of tissue sensitivity may be playing a role. Whether or not the child develops an increase in histamine sensitivity is not known. It must be kept in mind in mak-

ing a comparison, that in man the histamine content of tissue is much greater than in the mouse, and that normally, sensitivity is also much greater. In the mouse, the tissue content is so low that the increased sensitivity can not be due to an additive amount of histamine released from the tissue. Whether or not the reaction is due to altered tissue susceptibility, vascular changes, a stress factor or some other factor must await further investigation. An explanation for the reaction in the mouse might help to elucidate the nature of this infection in man.

The resistance of the recovered mouse, to an intracerebral inoculation of *H. pertussis* provides further justification for the use of the latter route in testing the protective potency of pertussis vaccines in mice(6).

Summary. Mice inoculated intranasally with a culture of *H. pertussis* developed lung infections from which more than half of the mice survived. The average time of death ranged from 16 to 23 days. They developed a high degree of sensitivity to histamine comparable to that induced by pertussis vaccine, but it was slower in developing and it persisted much longer. A high percentage of the mice that died from histamine shock showed gross lung lesions and *H. pertussis* was recovered from the lungs of the majority. Most of the mice that survived this infection were resistant to an intracerebral inoculation of *H. pertussis*.

6. National Institutes of Health, Minimum Requirements: Pertussis Vaccine, 1948.

Received April 11, 1951. P.S.E.B.M., 1951, v77.

Sulfhydryl Content of Normal Hemoglobin and Hemoglobin in in Sickle Cell Anemia.* (18683)

SIDNEY H. INGBAR AND EDWARD H. KASS.† (Introduced by Maxwell Finland)

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and Department of Medicine, Harvard Medical School.

Recent observations suggest that the abnormal physical and physiological properties of the erythrocytes in sickle cell anemia can be explained on the basis of abnormalities in their intracellular components. Evidence of a difference between the molecules of normal and of sickle cell hemoglobin has been obtained by electrophoresis(1). The increase in the viscosity of the whole blood of patients with sickle cell anemia which occurs on deoxygenation(2) has been reproduced in solutions of hemoglobin(3). The birefringence noted in sickled erythrocytes(4) is also a property of the tactoid aggregations which form when solutions of sickle cell hemoglobin are deoxygenated, and the tactoids themselves resemble in shape sickled erythrocytes(3).

Sickling *in vitro* of intact erythrocytes can be produced by a variety of reducing agents (5). Among these are cysteine, glutathione and BAL, which contain sulfhydryl (SH) groups(6). Sickling is inhibited by Hg^{++} and As^{+++} ions, iodoacetamide and o-chloromercuribenzoate, all of which are SH inhibitors(6,7). Thus, it appears that SH groups may be specifically concerned in the sickling phenomenon. It was of interest, therefore,

* Aided by a grant from the U. S. Public Health Service.

† Senior Fellow in Virus Diseases, National Research Council.

1. Pauling, L., *et al.*, *Science*, 1950, v111, 459.

2. Ham, T. H. and Castle, W. B., *Trans. A. Am. Physicians*, 1940, v55, 127.

3. Harris, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 197.

4. Sherman, I. J., *Bull. Johns Hopkins Hosp.*, 1940, v67, 309.

5. Daland, G. A., and Castle, W. B., *J. Lab. and Clin. Med.*, 1948, v33, 1082.

6. Thomas, L., and Stetson, C. A., Jr., *Bull. Johns Hopkins Hosp.*, 1948, v83, 176.

7. Kass, E. H. and Ingbar, S. H., Unpublished data.