in distribution patterns, and just as with Triton X-100, the triiodothyronine-I-131 became dissociated from the conventional transport proteins but with substantially smaller concentrations of detergent.

Triton X-100 or Tween 80, when dissolved in saline containing I-131-labeled-thyroxine or triiodothyronine and then applied directly to filter paper exhibited peaks of radioactivity, after electrophoresis, which approximated the zone of the gamma globulins of control sera. Hence, it was not possible to ascertain whether the nonionic agents in combination with radio-hormones migrated freely, or migrated as a complex in association with the gamma globulins. Furthermore, the possibility that the detergents might have acted by increasing the affinity between the gamma globulin fraction and the thyroid hormones directly could not be excluded.

Consideration might be given to the possibility that denaturation of thyroxine-binding globulin by detergent action could have resulted in the impairment of transport by the inter-alpha globulin fraction with consequent adsorption of the "loose" thyroxine-I-131 at the origin of the filter paper. This does not seem likely, however, for had there not been active binding of radiothyroxine by the gamma globulin complex, the displaced thyroxine-I-131 should have migrated in association with albumin. Furthermore, if surface-active agents acted solely by damaging thyroxine-binding globulin and thereby impairing the transport mechanism, the affinity between serum proteins and the labeled-hormones should be greatly reduced. The converse has been shown to obtain, however; plasma obtained from animals treated with detergents or plasma to which detergents were added had an augmented ability to bind triiodothyronine-I-131(1).

Conceivably, a similar mechanism of increased plasma binding with regard to cholesterol might be invoked to explain the hypercholesterolemia observed in animals shortly after administration of a nonionic surface active agent(2).

Summary. The conventional patterns of radioactivity observed after filter paper electrophoresis of serum containing I-131-labeled thyroxine or triiodothyronine were altered by addition of nonionic surface-active agents *in vitro*. Triton X-100 or Tween 80 added to serum interfered with the binding of T4-I-131 or T3-I-131 by TBG and resulted in the migration of radio-hormones in the zone of the gamma globulins. The concentration of radioactivity found in the region of the gamma globulins depended on the quantity of non-ionic detergent which had been added to the serum.

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Histamine Sensitivity in Mice of Different Ages after *Bordetella pertussis* Treatment or Adrenalectomy. (29592)

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The degree of histamine sensitization induced by *Bordetella pertussis* in mice is influenced by age(1,2,3). Thus, within a particular strain, young mice are less susceptible to this effect than older mice. For example, the LD_{50} of histamine diphosphate for one strain of 6-week-old *B. pertussis-treated* mice was found to be 54 mg/kg body weight, while it was only 17 mg/kg body weight for 12-week-old mice(1,4). The LD_{50} for unsensitized adult mice of the same strain was greater than 1000 mg/kg body weight(4).

TABLE I. Development of Histamine Sensitivity After *B. pertussis* Treatment or Adrenalectomy in RML Mice of Increasing Age.

		Normals			*B. pertussis*				
		Inoc with saline i.p. 4 days prior to challenge			Inoc with 40 μg HSF 4 days prior to challenge				Adrenalectomized 4 days prior to challenge, .5 mg hist
Age (wk)	Wt (g)†	12.5 mg hist*	25.0 mg hist	LD_{50} hist (mg)	.5 mg hist	2.5 mg hist	12.5 mg hist	LD_{50} hist (mg)	
3	13 ± 1.6	0/10‡	9/10	18.4	2/10	1/10	6/10	6.8	0/10
4	18 ± 1.9	0/10	7/10	20.5	2/10	3/10	4/8	5.5	0/15
5	22 ± 1.8	0/11	3/10	>25.0	3/10	5/10	7/10	2.5	1/12
6	23 ± 3.0	0/10	5/10	25.0	4/10	7/10	6/9	1.4	1/10
7	24 ± 2.9	1/10	4/10	>25.0	4/10	9/10	9/11	.9	0/10
8	25 ± 2.4	0/10	8/10	19.3	6/10	9/10	8/10	.6	1/11
9	27 ± 2.5	1/10	8/11	19.3	7/10	7/10	10/10	<.5	6/12
10	26 ± 2.7	0/10	5/10	25.0	8/10	8/10	6/10	.5	3/10
11	28 ± 3.4	0/10	6/10	22.3	5/10	8/10	9/10	.8	—
12	28 ± 2.8	1/10	5/10	23.3	6/10	8/10	9/10	.6	3/10
13	28 ± 2.5	0/10	7/10	20.5	4/10	10/10	8/10	.8	1/15
14	29 ± 2.5	0/10	6/10	22.3	8/10	10/10	9/10	<.5	6/11

* Challenge (mg histamine base)/mouse. † Mean ± S.E. ‡ Deaths/total challenged.

The 20-fold increase in histamine sensitivity shown by the 6-week-old mice indicated that they were already quite susceptible to the effects of *B. pertussis*. Kind(1) recommended that mice should be 14 g or over to insure maximum sensitivity to histamine after *B. pertussis* inoculation. However, there is considerable variation among strains of mice in the age or body size at which hypersensitization occurs. Munoz and Schuchardt(3) found strains which were still quite resistant to the effects of *B. pertussis* at 14 to 16 g body weight and in some of these strains adrenalectomy also failed to sensitize them(5). Gauthier *et al*(6) found that one strain of mouse became hypersensitive to histamine after administration of *B. pertussis*, but not after adrenalectomy.

Little is known about the effect of aging on the susceptibility of "resistant" strains to the histamine sensitizing effect of *B. pertussis* or adrenalectomy. This study presents results obtained with the Rocky Mountain Laboratory (RML) strain of mouse which is relatively resistant to sensitization at 3 to 5 weeks of age. The results indicate that with age these mice become susceptible to the histamine sensitization effect of either *B. pertussis* or adrenalectomy.

Materials and methods. Mice. Female mice of the RML strain were housed in groups of 5 in glass jars, 7 inches deep by 7 inches in diameter, containing beet pulp bedding and Purina Laboratory Chow.

Histamine sensitizing factor (HSF). HSF was prepared from *B. pertussis* cells as described previously(7).

Histamine. Histamine was given as histamine diphosphate (Nutritional Biochemical Corporation), but doses are expressed as histamine base.

Adrenalectomy. Bilateral adrenalectomies were performed as previously described(5). Mice were supplemented with 1% NaCl in the drinking water and allowed at least 24 hours to recover from the operation.

Sensitization and challenge procedures. HSF dissolved to the desired concentrations in 0.2 ml of physiological saline was administered intraperitoneally (i.p.) 4 days before challenge, while control mice received 0.2 ml of physiological saline. Challenge of either HSF inoculated or adrenalectomized mice consisted of an i.p. injection of the desired amount of histamine diphosphate dissolved in 0.2 ml physiological saline.

Results. The histamine sensitivity of normal, HSF-treated and adrenalectomized RML mice was determined for ages progressing from 3 to 14 weeks. Table I shows that the HSF-treated mice became increasingly sensitive to histamine from 3 to 9 weeks of age, while normal mice did not show any marked increase. The adrenalectomized mice also be-

TABLE II. Effect of Age on Histamine Sensitivity in RML Mice After *B. pertussis* Treatment or Adrenalectomy.

Histamine challenge (mg hist base/mouse)	4- to 5-wk-old mice			10- to 12-wk-old mice		
	Normals, saline i.p.	*B. pertussis*, 40 μg HSF i.p.	Adrenalx,* 1 day prior to challenge	Normals, saline i.p.	*B. pertussis*, 40 μg HSF i.p.	Adrenalx,* 1 day prior to challenge
	(4 days prior to challenge)			(4 days prior to challenge)		
.5	0/10†	0/10			5/10	5/11
1.0	0/10	0/10			10/10	7/11
2.0	0/20	3/19	0/10		8/10	
4.0	0/20	4/20	0/10			
8.0	0/20	4/20	1/10	0/10		
16.0	8/20	16/20	9/10	3/10		
32.0	19/20	17/19		10/10		
LD_{50} hist (mg)	18.5	10.2	11.3	19.5	.6	.7

* Adrenalectomized. † Deaths/total challenged.

came more sensitive beyond 9 weeks of age, but their sensitivity was not nearly as uniform as that of HSF-treated mice. The difference in the change in sensitivity between the young and older HSF-treated mice was quite dramatic. The 3-week-old mice became approximately 3 times more sensitive to histamine, while the 9-week-old mice became about 40 times more sensitive than respective normal control mice.

Another study was made on RML mice at 4 to 5 and 10 to 12 weeks of age with only 2-fold differences in the histamine challenges. Results of this study shown in Table II confirmed that young mice are quite resistant to the histamine sensitizing effects of either HSF or adrenalectomy while older mice are markedly sensitive.

Previous studies on histamine sensitivity in adrenalectomized mice were limited. Only 2 different doses of histamine had been used for challenge (0.5 and 1.0 mg) and the time between adrenalectomy and challenge had been 1 or 4 days. A more comprehensive study was made on the response of adrenalectomized mice to histamine. The results in Table III show that the response of these mice varies considerably, depending on amount of histamine injected and time elapsed between operation and challenge. The most striking result was the lower mortality after a 4.0 histamine challenge in comparison with the high mortality after 1.0 mg histamine. These findings are comparable to the results reported by Munoz *et al*(5,8).

TABLE III. Histamine Sensitivity in Adrenalectomized 8-Week-Old RML Mice.

Challenge (mg hist base per mouse)	Days between adrenalectomy and challenge		
	1	4	8
.5	2/10*	2/10	2/10
1.0	8/10	7/10	6/10
2.0	8/10	7/10	5/10
4.0	4/10	4/10	2/10
LD_{50} hist (mg)†	.8	.9	1.1

* Deaths/total challenged.
† Values do not include data for 4.0 mg challenge.

Discussion. Reasons for the striking difference among strains of mice to the histamine sensitizing effect of *B. pertussis* have not been found. A basic physiological difference between susceptible and resistant strains must exist. The present study indicates that this physiological difference is influenced by age and that in older mice the mechanisms involved in resisting the histamine sensitizing effects of HSF degenerate. The RML mouse which resists sensitization at 3 to 5 weeks of age becomes quite susceptible at 8 weeks and later. Knowledge of physiological changes which occur to make this mouse more susceptible to the action of HSF might lead to a possible explanation of the mechanism of action of HSF. The similarity between the effects of *B. pertussis* and of adrenalectomy on histamine sensitization has long been recognized(1,2,5). However, no direct effect of HSF on the adrenal gland or on circulating levels of adrenal hormones has been demonstrated(2). Concurrent development of histamine sensitivity in older mice after HSF

inoculation or adrenalectomy again indicates that some relationship exists between the two treatments. The decreased mortality in adrenalectomized mice receiving a histamine challenge of 4.0 mg in comparison with those receiving 1.0 mg is noteworthy. It is similar to the biphasic response reported by Munoz *et al* for *B. pertussis*-treated(8) and adrenalectomized mice(5), challenged with 8.0 mg histamine as opposed to those receiving only 0.5 to 2.0 mg. It suggests that mice may possess an inactive protective mechanism which can be activated with the proper stimulus. Possibly, extra-medullary chromaffin tissues may form part of this mechanism. It can be postulated that there are quantitative differences in the functional capability of these tissues among strains of mice and among ages within the same strain. The observations made by Gauthier *et al*(6) that one strain of mice failed to become sensitized to histamine after adrenalectomy, while becoming sensitive when inoculated with pertussis vaccine, might be explained on the presence, in that strain, of well developed extra-medullary chromaffin tissue. This is the most logical explanation at present.

Summary. The Rocky Mountain Laboratory strain of mouse which is resistant to the effect of histamine sensitizing factor from *Bordetella pertussis* (HSF) at 3 to 5 weeks of age became susceptible to this effect by the age of 8 weeks. The LD_{50} of histamine in HSF inoculated 3-week-old mice was 6.8 mg per mouse (524 mg/kg), while in 9-week-old mice it was less than 0.5 mg per mouse ($<$18.5 mg/kg). The LD_{50} of histamine in the 3-week-old control mice was 18.4 mg per mouse (1417 mg/kg), while in 9-week-old animals it was 19.3 mg per mouse (715 mg/kg). Development of histamine sensitivity after adrenalectomy was also found to be affected by age: LD_{50} of histamine for 4- to 5-week-old mice was 11.3 mg per mouse while it was only 0.7 mg for 10- to 12-week-old mice. Some physiological change related to aging must occur in these mice to bring about the change in susceptibility to histamine sensitization after HSF inoculation. This change appears to be related to the adrenal hormones, but the relationship is not clearly understood.

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Hemadsorption to Sections of Virus-Infected Tissues. (29593)

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Some viruses cause red blood cells (RBC) to adhere to the surface of infected cells in tissue cultures. An extension of this hemadsorption phenomenon to microtome sections of infected tissues would possess certain advantages both for diagnostic and investigative use. This paper describes the application of a slide-chamber technique to this problem, using tissues infected with the viruses of vaccinia, Newcastle disease (NDV), and influenza.

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