Table 1. Genetic classification of Mdh-1 phenotypes in *Mus musculus*.

Parental Combinations	Phenotypes	Progeny phenotype: A (slow) ♂	A (slow) ♀	AB (hybrid) ♂	AB (hybrid) ♀	B (fast) ♂	B (fast) ♀
SJL/J × SJL/J	A × A	6	4	0	0	0	0
C57BL/6J × C57BL/6J	B × B	0	0	0	0	6	4
SJL × C57BL/6J (F_1)	A × B	0	0	4	6	0	0
F_1 × F_1 (F_2)	AB × AB	7	20	21	24	3	15
F_1 × SJL/J (BC)	AB × A	6	3	2	5	0	0
F_1 × C57BL/6J (BC)	AB × B	0	0	6	3	3	1

Table 2. Mdh-1 phenotypes in different inbred strains of *Mus musculus*.

A (slow)			B (fast)		
SJL/J	A/HeJ	BALB/cJ	C57BL/6J	C58/J	CBA/J
LP/J	CE/J	SWR/J	C57BL/KsJ	AKR/J	CBA/CaJ
MA/J	C3HeB/FeJ	BuB/BrJ	C57BL/6J-Ob	ST/bJ	NZB
PL/J	129/J	LG/J	C57Br/cdJ	P/J	
DBA/1J	RF/J	DE/J	C57L/J	SEA/GrJ	
DBA/2J	DW/J				

were detected in inbred or wild mice.

Electrophoretic patterns of liver and kidney extracts from heterozygotes suggest that the Mdh-1 enzyme is a tetramer. If the enzyme were dimeric and the subunits could be distinguished electrophoretically, heterozygotes would exhibit three enzyme bands: two bands composed of parental-type subunits (*aa* or *bb*) and a single hybrid enzyme of intermediate mobility with the *ab* configuration. The relative activities of the three bands would conform to a ratio of 1 : 2 : 1. If, however, the enzyme were a tetramer, then the expected hybrid phenotype would consist of five bands of activity composed of the following subunit combinations: *4a*; *3a1b*; *2a2b*; *1a3b*; *4b*. The staining intensity of the hybrid phenotype would express a 1 : 4 : 6 : 4 : 1 ratio which would be consistent with expansion of $(a + b)^4$.

Five distinct bands demonstrating the expected activity ratio were reproducibly observed in all hybrid animals (Fig. 1, channels 1, 3, 5); these bands provide evidence for a tetrameric molecule (*6*). In some hybrid animals the most cathodally migrating enzyme band (band 5, channels 1, 3, 5) stained faintly in the *p*H 6.3 bridge and 6.7 gel buffer systems, and they may not be visible on the zymogram reproduction. At *p*H 7.0 and 7.4, band 5 stained more intensely and appeared more stable, but under these electrophoretic conditions the isozymes migrated less cathodally, and bands 1 and 2 were obscured by the gel origin. A five-band isozyme pattern was not observed when crude kidney and liver homogenates from A (slow) homozygotes were mixed with extracts from B homozygotes.

Hsu and Lardy (*3*) reported that the molecular weight of pigeon liver NADP-MDH was 280,000, and from NADPH binding and alkaline dissociation they estimated the number of subunits to be four. This supports the tetrameric structure of mouse Mdh-1. The catalase tetramer (*7*), having a molecular weight of about 240,000, has also been studied with genetic variants and electrophoretic techniques similar to those reported here (*8*). The catalase hybrid phenotype, like the Mdh-1 hybrid, consisted of five enzyme bands typical of a tetramer.

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1. The following abbreviations are used: NADP and NADPH, nicotinamide adenine dinucleotide phosphate and its reduced form; MDH, malate dehydrogenase.
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Hereditary Autoimmune Thyroiditis in the Fowl

Abstract. *In the Cornell C strain of White Leghorn chickens, thyroiditis appeared spontaneously. It was characterized by phenotypic changes due to a decrease of thyroxine, histological damage to the thyroid gland, and the presence in the serum of antibodies to thyroglobulin. Susceptibility to the disease is genetically inherited. Hormonal bursectomy (androgen given to the embryo) suppresses the development of thyroiditis.*

Hereditary hypothyroidism in the domestic fowl (*1*) is characterized by changes in the thyroid glands, which are somewhat similar to those seen in chronic thyroiditis (Hashimoto's disease) of man. In chickens, the phenotypic expression of this genetic variation consists of obesity, a skeletal structure somewhat smaller than normal in size, long silky feathers, and poor laying ability. Such characteristics are similar to those resulting from thyroidectomy and are correlated with a deficiency of thyroxine. Histologically the thyroid glands become infiltrated with lymphoid cells. The latter change begins about 2 weeks after hatching and subsequently becomes more intense. These symptoms (*1*, *2*) occurred spontaneously in the Cornell C strain of White Leghorn chickens (a closed flock since 1935). Selective breeding for this obese-hypothyroid trait demonstrated the hereditary nature of the disease and increased the incidence in females from less than 1 percent to over 90 percent, and in males from zero to over 80 percent. These higher percentages occurred in the latest two generations and represented observations on more than 400 chickens of each sex. Changes in the phenotype and physiology can be reversed by providing iodinated casein, a thyroxine-like substance, in the diet at a level of 0.011 percent.

Chronic thyroiditis in man appears to be associated with the development of antibodies specific for thyroglobulin. Witebsky and Rose (*3*) produced a similar disease in rabbits by autoimmunization. The exact mechanism of tissue damage has not been established. Spontaneously occurring thyroiditis in the fowl may present an interesting opportunity for exploration of many aspects of this disease.

The bursa of Fabricius in the chicken controls the development of humoral

Table 1. The effect of hormonal and surgical bursectomy on the incidence of clinical hypothyroidism in a strain of hypothyroid chickens.

Treatment	Obese-hypothyroid trait			
	Females (No.)		Males (No.)	
	Tested	Positive	Tested	Positive
Classified at 13 weeks				
Androgen	18	1*	13	0
None	19	19	21	20
Classified at 6 to 8 weeks				
Surgery	21	12	30	10
None	24	22	24	17

* This one female did not show enlargement of its comb at hatching, indicating that it had not been exposed to exogenous androgen.

antibody, and the thymus in the chicken controls the development of immune reactions mediated by lymphoid cells (*4*). To study various immunocompetent cells in spontaneous thyroiditis of the fowl, a hormonal bursectomy was performed by placing androgen in direct contact with the embryonating egg (*5*). In about 40 percent of the birds this treatment affects the thymus also (*6*). Pedigreed eggs from selected breeders of the obese-hypothyroid stock (*1*) were incubated for 3 days. At that time, half of the eggs from each dam were dipped, to a depth of 3 cm for 5 seconds, in absolute ethanol containing testosterone proprionate (3 g per 100 ml), the other half being in alcohol alone. None of the chicks from eggs treated with androgen (25 females and 44 males) developed symptoms of hypothyroidism. Of the untreated controls, 71 of the 77 females and 44 of the 59 males did develop the characteristic obese-hypothyroid trait.

Table 2. Tests on obese-hypothyroid female chickens.

Chickens (No.)	Obesity* (52 wk)	Hemagglutination titer†	Pathology of thyroid‡
PG 72	++	80	+++
PG 89	++	5120	++++
IG 1392	++	640	++
IG 1396	++	640	NT§
PG 70	++	160	++
IG 1389	++	2560	NT
PG 91	+	1280	+
PG 68	++	2560	++++
PG 69	++	40	NT
IG 1385	++	80	+++

* Phenotypic classification of obesity, + (moderate), ++ (severe), was made on the basis of excess subcutaneous fat and appearance of long silky feathers. A very similar classification was observed at 6 to 8 weeks of age in this group. † Hemagglutination titers are reported on the final bleedings at the time of autopsy (14 months of age). ‡ Histological appearance of thyroid at autopsy was recorded as + to ++++ indicating alteration varying from focal infiltration of lymphoid cells to extensive infiltration in many sections of the gland and almost complete destruction of all follicles. Both glands were similarly affected. § No test. Insufficient tissue for evaluation.

The thyroids of the hormonally bursectomized birds were approximately normal in size and variability. Those from the untreated obese chickens were generally smaller, and were more variable. At 14 weeks, the coefficients of variation for weight of thyroids from the obese females and males were 182 and 85 percent while those for the bursectomized birds were 30 and 49 percent, respectively. The thyroids from the treated chicks showed normal histology; the thyroids from the untreated controls showed varying degrees of lymphoid cell infiltration and the weight of the gland often reflected this pathology rather than the amount of thyroid tissue present.

These data indicate that the typical symptoms and thyroid pathology of the hereditary hypothyroidism can be prevented by hormonal bursectomy. Verification of the effect of bursectomy upon the expression of the trait was obtained as follows. Both the administration of testosterone to older embryos (12 to 13 days), which results in a failure of the bursa to develop but without the adverse effect on viability of the chicks (*7*), and surgical bursectomy performed on the 1st and 2nd day after hatching significantly affected ($P < .02$) the development of symptoms of hypothyroidism (Table 1).

The clinical and pathological changes in the obese-hypothyroid chickens and the modification of symptoms by hormonal as well as surgical bursectomy suggests that possibly we are dealing with a spontaneous thyroid disease resembling that seen in the human. To obtain additional evidence, tests for serum antibody to thyroid were made by the hemagglutination procedure with human red blood cells treated with tannic acid and coated with chicken thyroglobulin (*3*). Normal agglutinins for human red blood cells, which occur occasionally in chicken serums, were absorbed prior to testing for thyroid antibody.

Circulating antibodies against chicken thyroglobulin varied in titer from 40 to 5120 in the serums of ten randomly selected, untreated obese chickens showing the characteristic phenotypic trait (Table 2). The serums of over 100 normal chickens from several strains and various ages did not reveal any thyroglobulin antibodies, with the exception of one serum which gave a titer of 20. Tanned human cells coated with extracts of chicken organs other than thyroid (*3*) (for example liver, kidney, spleen, and heart) failed to reveal any agglutination with the serums of obese chickens.

The specificity of the agglutination was ascertained by the hemagglutination-neutralization test. Only thyroid extract and no other organ extract inhibited the agglutination of tanned red blood cells coated with chicken thyroid extract. Complete inhibition was observed when the thyroid extract was diluted as high as 1:320.

The serums from obese chickens were tested also with the Ouchterlony technique. A single strong line of precipitation was observed frequently after 24 hours with dilutions of the thyroid extract up to 1:250.

Thus, the development of spontaneous thyroiditis in the chicken provides a tool for studying several aspects of spontaneous autoimmune disease. In this respect the disease might resemble that found in the famous NZB strain (New Zealand black mice) as discovered by Bielschowsky, Helyer, and Howie (*8*), and which is generally considered a prototype of spontaneously developing autoimmune disease in animals. However, like Bielschowsky *et al.* we must learn whether we are dealing with a genetically controlled constitutional disease of spontaneous origin or whether a virus might be the eliciting factor.

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