reached previously[4-6] by showing the ability of other saponins to form complexes with cholesterol in monolayers of cholesterol or in virus and red blood cell membranes.

Having established the relationship between the presence of medicagenic acid in lucerne saponins and their hemolytic activity, it was of interest to examine, whether there are certain structural features in medicagenic acid, which are essential to its ability to haemolyze red blood cells. Comparison of the structures of the various soyasapogenols[7-9], with that of medicagenic acid shows that, the most outstanding difference is the presence of two COOH groups in the latter[10], which are absent from the soyasapogenols. Attempts have therefore been made to examine the hemolytic activity of free medicagenic acid and of its derivative in which the free carboxyl groups are blocked. Medicagenic acid was isolated by semi-preparative thin layer chromatography[2] from an acid hydrolysate of S_r ; a sample of it was converted to its dimethylester with diazomethane as described by DJERASSI et al.[10].

As shown in the Figure, on blocking the free carboxyl groups, medicagenic acid loses practically all of its hemolytic activity. Thus it can be concluded that, in the case of lucerne saponins, the presence of free carboxyl groups of medicagenic acid is essential to their ability to hemolyze red blood cells[11,12].

Zusammenfassung. Die aus Wurzeln von Luzerne isolierten Saponine wirken stärker hämolytisch als diejenigen in den grünen Teilen dieser Pflanze. Die hämolytisch wirksameren Saponine sind reicher an Medicagensäure und es wird angenommen, dass diese für die hämolytische Wirkung der Saponine verantwortlich ist. Die Anwesenheit von 2 Carboxylgruppen in der Medicagensäure scheint für ihre hämolytische Wirkung von ausschlaggebender Bedeutung zu sein, da der Dimethylester dieser Säure nicht mehr hämolytisch wirkt.

B. GESTETNER, S. SHANY and Y. ASSA

Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot (Israel), 26 June 1970.

[4] A. M. GLAUERT, J. T. DINGLE and J. A. LUCY, Nature, Lond. *196*, 953 (1962).
[5] R. R. DOURMASHKIN, R. M. DOUGHERTY and R. J. C. HARRIS, Nature, Lond. *194*, 1116 (1962).
[6] A. D. BANGHAM and R. W. HORNE, Nature, Lond. *196*, 952 (1962).
[7] A. MEYER, O. JEGER and L. RUZICKA, Helv. chim. Acta *33*, 672 (1950).
[8] H. M. SMITH, J. M. SMITH and F. S. SPRING, Tetrahedron *4*, 111 (1958).
[9] D. WILLNER, B. GESTETNER, D. LAVIE, Y. BIRK and A. BONDI, J. chem. Soc. *S 1*, 5885 (1964).
[10] C. DJERASSI, D. B. THOMAS, A. L. LIVINGSTON and C. R. THOMPSON, J. Am. chem. Soc. *79*, 5292 (1957).
[11] Thanks are due to Prof. C. DJERASSI for a generous gift of a sample of medicagenic acid dimethylester.
[12] This work was supported by Grant No. FG-Is-246 from the U.S. Department of Agriculture.

Strain Difference in Sex Ratio Response of Mice to Lactate Dehydrogenase Virus Infection[1]

Initially, one of us[2] reported a significant alteration in the sex ratio of weanlings born to C57BL/Fg mice inoculated, at 10–19 days before conception, with the lactate dehydrogenase (LDH) virus[3]. Then, data were obtained to suggest that the alteration in sex ratio stems from a response of the infected C57BL/Fg sire to the characteristic increase in plasma LDH activity[4]. In this paper, we wish to describe results obtained in related studies with mice of the BALB/cDg strain which have a spontaneously low sex ratio.

Materials and methods. Animals. BALB/cDg male and female mice, 4–6 months old, were bred in the research colonies of The Jackson Laboratory. Strain C57BL/Fg animals were supplied by Dr. F. H. J. FIGGE, University of Maryland School of Medicine.

LDH determinations. Blood was collected by tail bleeding; plasma LDH activities were measured by the method described elsewhere[5].

Sex ratios. Adult BALB/cDg mice were mated (5 females and 2 males per cage) at intervals of 10–14 days after an i.p. injection of mouse plasma (0.1 ml/animal) containing $10^{7.0}$ ID_{50}/ml of the LDH virus. Those females which became pregnant were isolated in separate cages; the number of babies born to each was recorded at birth. Thereafter, mother and progeny were treated as described previously[2] except that all dead offspring were examined for sex as well as gross pathologic lesions. Control animals received an i. p. injection (0.1 ml/mouse) of phosphate-buffered saline (PBS), pH 7.2.

Spermatozoa. Vasa deferentia from normal and 14-day-infected male mice (BALB/cDg and C57BL/Fg) were stripped into 1.0 ml of cold PBS in separate Syracuse watch glasses. After 3 washings, each sperm sample was resuspended in 0.5 ml of PBS, and then frozen and thawed before centrifugation at 2500*g* for 20 min at 0 °C. The resulting supernatant extract was assayed spectrophotometrically for LDH activity.

Results and discussion. As shown in Table I, whereas the sex ratio of offspring born to noninfected BALB/cDg parents was 41:59, that observed among the progeny of matings between LDH virus-infected animals was 49:51 (chi square, 2.45; P 0.12). This finding proved of interest because it suggested a response in sex ratio opposite to the direction reported in previous studies with C57BL/Fg mice[2]. Accordingly, additional experiments were undertaken to investigate certain factors which might be of significance.

The first experiment was designed to see if the plasma LDH levels of noninfected and infected BALB/cDg mice were the same as or distinct from those recorded among comparable groups of C57BL/Fg animals. The results, summarized in Table II, indicate that they are similar. Therefore, the difference in sex ratio response cannot be explained on the basis of a strain difference in: 1. the

[1] Supported by a grant from the Maryland Division of the American Cancer Society.
[2] C. G. CRISPENS JR., Nature, Lond. *214*, 819 (1967).
[3] V. RILEY, F. LILLY, E. HUERTO and D. BARDELL, Science *132*, 545 (1960).
[4] C. G. CRISPENS JR., Experientia *25*, 1197 (1969).
[5] C. G. CRISPENS JR., J. natn. Cancer Inst. *34*, 331 (1965).

Table I. Alteration of the sex ratio of offspring born to BALB/cDg mice infected with the lactate dehydrogenase virus

Items	Mice Experimental[a,b]	Control[c]
Litters	36	41
Babies	228	268
Babies/litter	6.3	6.5
Weanlings	126	140
Weanlings/litter	3.5	3.4
Male offspring	85	81
Female offspring	88	117
Male: female sex ratio (%)	49:51[d]	41:59

[a] Infected for 10 to 19 days before conception. [b] A total of 55 offspring were not sexed. [c] A total of 70 offspring were not sexed. [d] P 0.12.

Table II. Comparison of plasma lactate dehydrogenase (LDH) levels in the BALB/cDg and C57BL/Fg strains of mice

Treatment	No. of mice tested per group	Plasma LDH (units/ml)			
		BALB/cDg		C57BL/Fg	
		Range	Mean	Range	Mean
Infected[a]	35	2400–5700	4150	2400–5400	4050
Noninfected	50	200–1200	700	300–1300	750

[a] Plasma samples collected 1 week after an i.p. injection (0.1 ml/mouse) of $10^{5.0}$ ID_{50}/ml of the LDH virus.

normal level of plasma LDH; or 2. the degree of increase in enzyme activity following infection with the LDH virus.

Table III shows the results obtained in the second experiment. It will be noted that spermatozoa LDH levels in normal BALB/cDg males were 3 times greater than those in infected animals. However, enzyme levels in similar preparations from infected and noninfected C57BL/Fg males were essentially the same. These data, then, may be said to offer partial support for the hypothesis that the alteration in sex ratio is related to the level of spermatozoa LDH. Beyond this, and until such time as further information becomes available, we can only suggest that alternative explanations also be explored.

Table III. Comparison of lactate dehydrogenase (LDH) levels in spermatozoa from normal and infected mice of the BALB/cDg and C57BL/Fg strains

Treatment	No. of mice tested per group	Spermatozoa LDH (units/ml of extract)			
		BALB/cDg		C57BL/Fg	
		Range	Mean	Range	Mean
Infected[a]	15	110–630	275	120–550	285
Noninfected	14	390–1660	775	110–730	275

[a] Sperm samples were collected 2 weeks after an i.p. injection (0.1 ml/male) of $10^{6.5}$ ID_{50}/ml of the LDH virus.

Zusammenfassung. Durch Infektion mit LDH-Virus wird bei Mäusen, deren Nachkommen normalerweise ein Geschlechtsverhältnis < 1 aufweisen, ein solches von 1 erreicht. Da sich die Infektion der Eltern beider Stämme nicht in einer unterschiedlichen Zunahme der Serum-LDH, wohl aber in einer solchen der Spermien-LDH auswirkt, wird angenommen, dass die LDH-Aktivität der Spermien sich in einer zwar noch unbekannten Weise auf das Geschlechtsverhältnis der Nachkommen auswirkt.

CH. G. CRISPENS JR. and W. K. WHITTEN

Department of Biology, The University of Alabama in Birmingham, Birmingham (Alabama 35233, USA), and The Jackson Laboratory, Bar Harbor (Maine 04609, USA),
24 July 1970.

A New Type of Acid Phosphatase from Rat Testis

At least three enzymatically distinct forms of acid phosphatase have been separated from various mammalian tissues[1–3]. They have different substrate specificities, different responses to inhibitors and different tissue and subcellular localizations. Histochemical studies have indicated that acid phosphatase is mainly associated with lysosomal particles[4]. Other sites of reaction are cisternae of smooth endoplasmic reticulum[5] and Golgi apparatus[6,7]. Similar localization has been demonstrated for acid phosphatase in testicular Leydig[8] and Sertoli cells[9] and additionally in acrosomes of spermatozoa[10,11].

The present study describes four distinct acid phosphatases in rat testicular tissue with special emphasis to a new soluble enzyme not previously described in other tissues.

Material and methods. Adult albino rats were sacrificed by rapid decapitation, their testes and samples from 9 other tissues were taken and homogenized at 4 °C either in 0.25 M sucrose solution or in 0.02 M *Tris*-HCl buffer pH 7.5 with a glass homogenizer and teflon pestle. Samples in 0.25 M sucrose were centrifuged with $105{,}000 \times g$ for 1 h and the soluble and particle fractions were diluted with 0.02 M *Tris*-HCl, pH 7.5, for enzyme assays. Protein was determined by the method of LOWRY et al.[12].

Tissue samples homogenized in *Tris*-HCl buffer were sonicated for 30 sec (MSE Ultrasonic Disintegrator), applied on a DEAE-cellulose (Whatman DE 23) column (2×30 cm) and eluted with a continuous NaCl gradient (0–0.35 M) in 0.02 M *Tris*-HCl buffer pH 7.5. Aliquots of 5 ml were collected. The protein content was determined by the absorbancy at 280 nm.

Acid phosphatase activity of total homogenate, soluble and particulate fractions and chromatographic fractions were assayed at 37 °C with *p*-nitrophenyl phosphate