of the artificial vagina (*6*). Seminal plasma and spermatozoa were separated by centrifugation at 12,800*g*. Epididymides of rabbits were removed from the animals immediately after exsanguination, and the spermatozoa were washed out of the dissected organs. Both kinds of spermatozoa were washed thrice in physiological saline solution, counted in a counting chamber, and suspended in physiological saline solution in the desired concentration. Complement fixation tests (Kolmer method) were made with these suspensions as antigens and the sera of guinea pigs immunized either with rabbit seminal plasma or with washed seminal spermatozoa. Controls with normal guinea pig serum and with immune serum from guinea pigs against whole rabbit serum remained entirely negative. Details on the techniques employed have been reported (*2, 4*).

The data obtained by this highly sensitive method show that epididymal spermatozoa lack the strongly antigenic material present in seminal spermatozoa and in seminal plasma (see Table 1).

These findings should not be interpreted as a denial of antigenicity of the spermatozoa as they originate in the testes. In fact, the data of Henle and his associates (*7*) and of Voisin (*8*) and Freund (*9*) show that testicular spermatozoa are not devoid of antigenic properties, and those of Pernot (*3, 5*) provide evidence that such antigenic properties can still be discerned in seminal spermatozoa. The point that concerns us here is that spermatozoa take up antigenic material from the seminal plasma before or at the time of ejaculation. It would be of interest to obtain information on the chemical nature of these antigens and their role, if any, in the physiology of reproduction (*10*).

Table 1. Fixation of complement by guinea pig immune serum (diluted 1/10) and antigen. The antiserum and antigen controls, also antigens plus normal guinea pig serum and antigens plus anti-rabbit serum immune serum, remained negative. ++++, No hemolysis; ++, moderate (about 50 percent) hemolysis; −, complete hemolysis.

Antigen dilution	Antiserum	
	Anti-spermatozoa	Anti-seminal plasma
Spermatozoa from semen (4 × 10^6 cells/ml)		
1/1	++++	++++
1/2	++++	++++
1/4	++++	++++
1/8	−	++
1/16	−	−
Spermatozoa from epididymis (4 × 10^6 cells/ml)		
1/1	−	−
1/2	−	−
1/4	−	−
1/8	−	−
1/16	−	−
Seminal plasma (diluted 1/1000)		
1/1	++++	++++
1/2	++++	++++
1/4	++++	++++
1/8	++++	++++
1/16	−	−

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Influence of pH on the Toxicity of Nitrogen Mustard

Abstract. The experiments reported here demonstrate the unexpected finding that, in mice, the *p*H of nitrogen mustard solution at the time it is injected into the animal appears to determine the toxicity of the material. At certain doses, highly acid solutions of nitrogen mustard show no toxic effects, while alkaline solutions at the same dose are invariably lethal. From further experiments on the antitumor activity of acid solutions of nitrogen mustard, it is concluded that the toxic effect is separable from the antitumor effect.

In the course of an investigation of the mechanism of toxicity of nitrogen mustard [methyl bis (β-chloroethyl)-amine·HCl; HN2] several facts have emerged: (i) toxicity can be prevented by the administration of any of several compounds before injection of the mustard (*1*); (ii) toxicity can largely be prevented in dogs by prior splenectomy and the administration of norepinephrine just before injection of HN2 (*2*); such protection is not conferred by splenectomy in mice (*3*); and (iii) intraperitoneal injections of mice with saline homogenates of spleen, mixed with nitrogen mustard, were uniformly more toxic than the same dose of nitrogen mustard alone (*4*). At the LD_{50} of nitrogen mustard (in saline), mixtures of mustard and spleen homogenates were uniformly lethal. The LD_{50} for HN2 in saline, in mice, is approximately 4.0 mg/kg, injected intraperitoneally (*5*).

In analyzing the data on the toxicity of mixtures of nitrogen mustard and spleen homogenates, it was apparent that the *p*H of the mixture was substantially higher than the *p*H of the mustard in saline alone. To control this discrepancy, Ringer's solution was substituted for the saline. When this was done, the *p*H's of the mixture of mustard and spleen homogenates and of the mustard in Ringer's solution were roughly the same, usually about 6.8. At this higher *p*H, the mustard was more toxic than it had been at the lower *p*H, and it was nearly as toxic as the mixtures of mustard and spleen homogenates. Therefore, a study of the effect of *p*H upon the toxicity of nitrogen mustard was started (*6*).

Male mice of the Swiss-Webster and C57Bl strains were used. All were healthy young animals maintained on a balanced ration with free access to food and water. Each animal was weighed just prior to injection, the dose of nitrogen mustard being based on its weight. At each *p*H, and for each dose, groups of 10 to 25 animals were used. To date, more than 3000 normal mice have been used in this toxicity study. For most of the work, intraperitoneal injections were used. For single doses 1.0 ml was given, and for fractionated daily doses the mustard was given in 0.5 ml of solution. In separate experiments the material was given intravenously in 0.2 ml of solution.

Nitrogen mustard (methyl bis (β-chloroethyl) amine·HCl; HN2], supplied commercially as Mustargen and diluted for injection (10.0 ml of saline or water per 10.0 mg of HN2) was found to have an initial *p*H of 4.7. When HN2 was diluted further for injection in mice, the *p*H of the material was usually about 5.2. To vary this *p*H, stock solutions of saline were made up with HCl or NaOH so that, upon addition to these solutions of HN2, a final *p*H of 2.0, 4.0, 6.0, 8.0, or 10.0 resulted. Several different doses of HN2 at each *p*H were used; through careful manipulation, the final *p*H's of the injected solutions within each *p*H group were quite close, in spite of tenfold differences in the concentration of HN2. All the solutions were injected within 60 seconds of mixing. An aliquot was taken, and the *p*H of each solution was measured thereafter in a Beckman model G *p*H meter.

Experiments were performed with doses of nitrogen mustard which ranged from 2.25 to 20.0 mg/kg, injected intraperitoneally as a single dose. In a few experiments the intraperitoneal dose was given as five consecutive daily injections, the total amounts ranging

Table 1. Percentage survival after single intraperitoneal injection of various doses of HN2 in strain C57B1 mice.

Survival (%) at various doses (mg/kg)				
3.0	5.0	7.5	10.0	20.0
		pH 2.2		
100	83	30	4	0
		pH 4.0		
92	0	0	0	0
		pH 5.2		
92	17	0		
		pH 7.2		
66	0			
		pH 8.9		
58				

from 5.0 to 9.0 mg/kg. In other groups doses of 3.0 to 4.0 mg/kg were given intravenously.

At every level at which it was tested, and regardless of the route by which it was given, nitrogen mustard was less toxic to mice at *p*H 2.0 than at any *p*H above this level. At low doses (2.25 to 2.75 mg/kg), deaths occurred only in animals that had received the material at *p*H 6.5 to 9.0. At high doses (7.5 to 10.0 mg/kg), only the animals that had received the material at *p*H 2.0 to 2.5 survived. Above 10.0 mg/kg, single doses of nitrogen mustard were lethal, regardless of *p*H. The dramatic effect of *p*H upon toxicity was more clearly seen at intermediate levels, in the range of 3.0 to 7.5 mg/kg.

Table 1 summarizes results in one experiment in which nitrogen mustard was given in a single injection at five different levels and five *p*H ranges. A dose of 3.0 mg/kg produced no deaths at *p*H 2.2 and deaths at *p*H 8.9 of nearly 50 percent. A dose of 5.0 mg/kg was lethal at *p*H 7.2, highly toxic at *p*H 5.2, and only slightly so at *p*H 2.2, with 83-percent survival at this low *p*H. At 7.5 mg/kg, which is above the accepted LD_{00} for mice, 30 percent of the animals that had received doses at *p*H 2.2 survived; there were no survivors at this dose at a higher *p*H.

The effect of hydrogen ion concentration upon toxicity is further illustrated in Fig. 1. It may be seen that with single doses of nitrogen mustard of 4.0 and 6.5 mg/kg there was little toxicity at *p*H 2.0, a substantial amount of toxicity at *p*H 5.0, and death in every case at *p*H 8.0 to 8.4.

Fractionation of the dose of nitrogen mustard decreased the toxicity of the material at the three *p*H ranges and two doses illustrated in Fig. 1. The pronounced effect of *p*H upon toxicity was again seen, and at total doses of HN2 of up to 8.75 mg/kg, no deaths were seen in groups that had received the material at *p*H 2.0. The same dose in a single injection, even at *p*H 2.0, was lethal for more than 75 percent of the injected animals. This dose (8.75 mg/kg) at *p*H 10, whether divided or given in a single injection, was invariably lethal.

With intravenous administration the striking effect of *p*H upon the toxicity of HN2, which had been repeatedly demonstrated with intraperitoneal injections, was again found. The toxic effects of nitrogen mustard were observed sooner after intravenous injection, and animals that had received 4.0 mg of HN2 per kilogram at *p*H 8.4 died within 3 to 4 days; with the same dose at *p*H 2.0 there were no deaths. With a dose of 4.0 mg/kg at *p*H 5.6, 40 percent of the injected mice died. With this dose of nitrogen mustard, given intraperitoneally to other mice (Fig. 1) there was the same range of toxicity, but with fewer deaths at high *p*H; deaths, when they occurred, came later with intraperitoneal injections than they did with intravenous injections. This finding is similar to many others that have been reported, which have shown that a dose of nitrogen mustard given intravenously is more toxic than the same dose given intraperitoneally (*5*).

The important effect of hydrogen ion concentration upon the toxicity of nitrogen mustard, which has been briefly described here and which was noted earlier by Boyland (*7*) is not easily understood. The body should be more than able to buffer the injected material. The possibility that the *p*H effect was peculiar to intraperitoneal injections, where buffering might be slow, was disproved by the finding that low *p*H has exactly the same protective effect against nitrogen mustard toxicity after intravenous injection.

In attempting to explain this phenomenon it was apparent that the entire sequence of chemical reactions of nitrogen mustard in vivo was affected by the *p*H at the time of injection, whether acid, neutral, or alkaline. The buffering action of blood was, although presumably almost instantaneous, insufficient to overcome this *p*H effect. Two possible explanations for the *p*H effect on nitrogen mustard toxicity are as follows. (i) Low *p*H prevents, and high *p*H induces, the rapid formation of toxic derivatives of nitrogen mustard in the solution of nitrogen mustard itself. Conversely, low *p*H may induce formation of a nontoxic mustard derivative. There are no data available to support this explanation. (ii) More probably, low *p*H prevents the formation, in vivo, of certain toxic products of the interaction of nitrogen mustard and some body constituent or constituents, while allowing the formation of other, nontoxic products. By contrast, high *p*H of injected mustard appears to change these reactions in the direction of formation of toxic product or products. This apparent mutability of the in vivo reactions of nitrogen mustard has significance in the elucidation of the mechanism of action of the drug. It was of great importance to determine whether such alterations affected the antitumor activity of nitrogen mustard.

The antitumor activity of highly acid

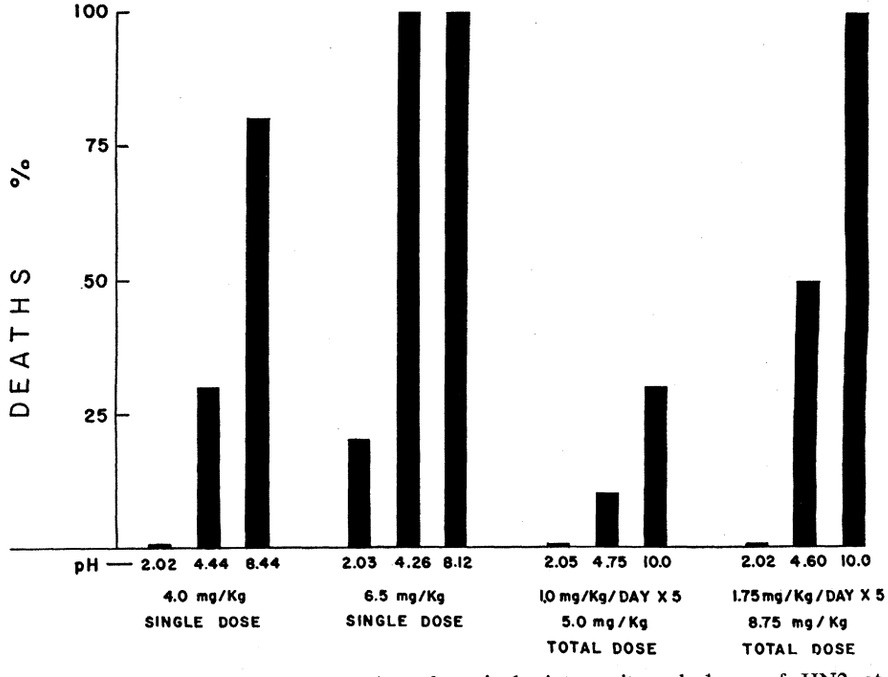

Fig. 1. Deaths in Swiss-Webster mice after single intraperitoneal doses of HN2 at various *p*H's, and after multiple daily doses of HN2, given intraperitoneally.

preparations of nitrogen mustard has been assayed in Swiss-Webster mice with Ehrlich ascites tumors. In repeated experiments, in each of which 300 mice were used, all untreated tumor-bearing animals died within 16 days (average 13.5 days), while mice that had received HN2 at *p*H 2 all lived more than 20 days, with average survival of 27 days, and some remain alive, without ascites, beyond that time.

It appears, therefore, that extremely low *p*H, while reducing the toxic effects of the nitrogen mustard in mice, does not interfere with the antitumor activity of the drug (*8*).

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Emphasis on Holotype (?)

Abstract. The description of new species should not be confined to physical description of a holotype. One specimen cannot include all characters or be typical of any taxon. The holotype serves only a nomenclatural function and might also be termed the name-bearer (nomenifer) to avoid confusion of "type specimen" with "typical specimen."

Shenefelt protests about vague, indefinite species descriptions, made ambiguous "deliberately," to cover a range of variation inherent in an abstract group concept (*1*). He says: "The purpose of a description is to convey a concept of the object under scrutiny as clearly as possible by means of words, pictures, or diagrams." He recommends that the holotype specimen be described relatively exactly and that the range of specific variation be discussed with reference to the holotype description. In this manner physical and abstract concepts would be differentiated more easily, and the functions of description would be served more effectively.

It is appropriate to protest about the quality of taxonomic descriptions in many fields of biology. Many taxonomic descriptions are poor for want of adequate concern about organization and content. Scientific authors seem to have difficulty in visualizing means of being helpful to readers. Shenefelt's emphasis on description of the physical holotype is not justified, however, from the standpoint of the basic objectives in taxonomy.

It has been emphasized repeatedly, for the benefit of plant taxonomists, at least, that the nomenclatural type (holotype) of a species is not to be confused or implicated in anyone's concept of what is "typical" for a taxon. A nomenclatural type is simply *the specimen*, or other element, with which a name is permanently associated. This element need not be "typical" in any sense; for organisms with a complicated life cycle, it is obvious that no single specimen could physically represent all the important characteristics, much less could it be taken to show many features near the mean of their range of variation. Consequently, an exact description of the holotype specimen leads us exactly nowhere in the process of discovering "modes," "means," or other "norms" typical of species.

Some approach to the problem of variation may be made by biometric analysis, and this information is pertinent for taxonomic description. However, descriptive matter is concerned *only* with more precise indication of the nature of the abstract group concept (species); this information has no bearing on, and never can have any essential relation to, selection or function of the nomenclatural holotype.

Often it has been noted that the term *type specimen,* in the sense of a nomenclatural type, is misleading because this "type" cannot be properly construed as being "typical." The terminology has been a source of misunderstanding, confusion, and misconception ever since the type "system" was introduced. The only function a nomenclatural type can serve is that of name-bearer. This function is perfectly mechancial in the technical manipulation of taxonomic nomenclature. Whatever may be said of its nomenclatural advantages, a discussion of the "type method" must always be phrased to avoid the misleading etymologic implications inherent in the term.

Perhaps if we were to speak of the name-bearer, or "nomenifer" method (L: *nomen*, name, + *ferre*, to bear), the proper implication would be more easily conveyed. Comprehension of the wholly arbitrary nature of the name-bearer specimen, however, is of the essence for understanding the meaning of "type method" in modern systematics. The term *type method,* usually properly used in the arbitrary sense, is now so entrenched in systematic literature that it would be most confusing to attempt to substitute any different term for it. However, if one wished especially to emphasize the name-bearing function, it might be permissible to insert the term *nomenifer* parentheticalally, following the term *holotype* ["holotype (nomenifer)"] at the place where the type specimen is designated after a species description. Evidently, judged by frequent recurrence of the misconception, something of this nature sometimes is needed to signify that the type specimen is not necessarily typical in any particular.

The concept of the "typical" representative is frequently misused in biology. When the term is used, a question always can be raised regarding the nature of the measuring operation and the adequacy of sampling. If the term is used, it should be carefully qualified; commonly better meaning is conveyed by avoiding use of the term *typical* and stating definite facts, rather than by providing a "typical" interpretation. Pre-Darwinian "typology," with implications harking back to fixity of species and special creation, is frequently involved with a "typical" concept of "type." Emphasis on description of the holotype, rather than on the concept of a species population, does not seem likely to improve our means of classifying organisms or in understanding other essential aspects of biologic problems (*2*).

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2. Publication authorized by the director, U.S. Geological Survey.

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Histochemical Distribution of Succinic Dehydrogenase in Bone and Cartilage

Abstract. Large amounts of succinic dehydrogenase have been demonstrated histochemically in osteoclasts and chondroclasts. The same enzyme was also found in the giant cell of giant cell tumors of bone. This distribution suggests a relation to bone and cartilage resorption.

Many histochemical studies of bone formation and resorption have appeared in recent years. These studies are of great interest, since processes take place in areas separated only by a few microns, and this makes the analysis of biochemical data of even very small samples very difficult.

It has been shown histochemically