constant in Eq. 4 and Eq. 5 are functions of temperature and the latter cannot be accurately evaluated until the data are extended to other temperatures.

Four oxidation-reduction reactions were studied at 700 ± 5°C and 800 ± 15 bars to demonstrate the use of the H_2 diffusion method in controlling f_{O_2}. These reactions are

I. $2Ni(s) + O_2(g) \leftrightarrows 2NiO(s)$

II. $3Fe_2SiO_4(s) + O_2(g) \leftrightarrows 2Fe_3O_4(s) + 3SiO_2(s)$

III. $6FeO(s, wüstite) + O_2(g) \leftrightarrows 2Fe_3O_4(s)$

IV. $2Fe(s) + O_2(g) \leftrightarrows 2FeO(s, wüstite)$

where s is a solid and g is a gas. Eugster and Wones (*5*, Table 1) give equations with a pressure term for the equilibrium oxygen fugacities of these reactions.

The reactions were studied by sealing the solid charges together with H_2O in platinum or palladium-silver capsules which were run at various values of f_{H_2}. Equilibrium was approached by causing the reaction to go one way or the other depending on whether the approach was from the direction of low f_{H_2} or high f_{H_2}. Table 1 gives these results in terms of the pressure of pure hydrogen gas at equilibrium, together with values of the oxygen fugacities calculated from the experimental results in this report for H_2-H_2O mixtures. These values of f_{O_2} are compared in Table 1 with values obtained, assuming ideal mixing for hydrogen-H_2O, and values calculated from equations given by Eugster and Wones (*5*).

For the case of ideal mixing, the fugacity of oxygen is given by

$$f_{O_2} = \left(\frac{P\nu^\circ_{H_2O}\nu^\circ_{H_2} - \nu^\circ_{H_2O} f_{H_2}}{K_f f_{H_2} \nu^\circ_{H_2}} \right)^2$$

where ν° is the fugacity coefficient at pressure P. The experimental data for H_2-H_2O mixtures were taken into account by calculating fugacities from activities (Fig. 1), and by using these with the equilibrium constant of formation for H_2O from Eq. 1.

In both cases the results are quite similar. Nonideality in the system H_2-H_2O is not strongly reflected in log f_{O_2}. The results for reaction III demonstrate that this method for controlling f_{O_2} can have high precision, potentially even better than that indicated.

This method permits simple control of f_{O_2} in hydrothermal experiments with good, and potentially excellent, precision and accuracy. There are several advantages over the "oxygen buffer" technique described by Eugster (*6*), and Eugster and Wones (*5*), perhaps the chief one of which is that P, T, and f_{O_2} may be varied independently and continuously. At the same time f_{H_2O} is determined, where previously some assumption was required for its evaluation. The activity of H_2O can be controlled by this method in hydrothermal studies which are not complicated by appreciable amounts of other components in the gas phase and in which hydrogen acts as an inert component in the gas (*7*).

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Hypersensitivity to a Synthetic Polypeptide: Induction of a Delayed Reaction

Abstract. *A multichain copolypeptide,* p*(Tyr,Glu)-pAla--*p*Lys, induced in guinea pigs a prolonged state of delayed hypersensitivity not associated with detectable circulating antibodies. Cross reactions of the delayed type were observed with chemically related polypeptides.*

The phenomenon of delayed hypersensitivity is assumed to be either an expression of "cellular" hypersensitivity independent of circulating antibody or to incur the obligatory participation of humoral antibodies of high affinity (*1*). For the second alternative an antibody ought to be able to form a stable union with the homologous antigen at concentrations of the uncombined antibody which are too low to be detected by available methods. Studies of delayed hypersensitivity to proteins have been summarized recently (*2*). It seems (*3*) that a functionally larger antigenic determinant controls the specificity of the delayed reactions than that which promotes the union of antigen with soluble antibody. The availability of chemically well-defined substances that would be capable of eliciting pure delayed hypersensitivity persisting for long periods would thus be of interest for the elucidation of the nature and specificity of this phenomenon.

We report the induction, in most guinea pigs tested with a multichain synthetic polypeptide, of a durable specific delayed hypersensitivity that was not associated with detectable circulating antibodies. The use of this antigen was suggested by recent reports (*4–6*) indicating the potent and specific antigenicity of certain synthetic polypeptides. When used alone, these polypeptides are capable of inducing both delayed and immediate sensitivity (*5*, *6*), whereas, when they are used as an antigen-antibody complex they are capable of inducing a pure delayed sensitivity in a few test animals (*6*).

The antigen used in this study, denoted *p*(Tyr,Glu)-*p*Ala--*p*Lys, is a multichain copolymer in which side chains of poly-DL-alanine, attached to the ε-amino groups of poly-L-lysine, were elongated with peptides containing L-tyrosine and L-glutamic acid (*4*). This substance elicited precipitating antibodies in rabbits (*4*), and immunological tolerance could also be obtained in these animals (*7*). Our two samples were 35 and 210, with average molecular weights of 35,000 and 33,200 respectively, and residue molar ratios of Lys:Tyr:Glu:Ala of 1 : 1.8 : 3.7 : 24 and 1 : 1.8 : 2.4 : 22, respectively. No differences were found in the immunological behavior of these samples.

A mixture (0.8 ml) of equal volumes of antigen in physiological saline and Freund adjuvant (Difco) was injected into the four footpads of white guinea pigs weighing 250 to 450 g. Intradermal tests were performed in the range of 30 to 2000 μg of antigen and were observed at 2, 4, 24, and 48 hours. Control skin tests with saline in experimental animals and skin tests with antigen in normal animals were all negative.

Negative skin reactions were observed in 17 animals which received 1.5 to 2.0 mg of antigen in the Freund incomplete adjuvant 10 days before testing. On the other hand, positive skin reactions occurred in 29 of 32 animals which received 1.5 to 10 mg of *p*(Tyr,Glu)-*p*Ala--*p*Lys in the Freund complete adjuvant (including killed

mycobacteria) 10 days before testing. Reactions which were visible by 18 hours were maximal after 24 hours. None were of the immediate or Arthus type. Neither was the test for anaphylaxis (*8*), in which 10 mg of antigen was given by intracardiac injection, followed by any symptoms. Both precipitin tests (*4*) and the passive cutaneous anaphylaxis tests (*9*) were negative. The latter procedure will detect as little as 0.2 μg of rabbit antibody to *p*(Tyr, Glu)-*p*Ala--pLys.

The histology of the delayed reaction is illustrated in Fig. 1. One day after the intradermal injection of the antigen there was a moderate infiltration of polymorphonuclear neutrophiles and mononuclear cells, most marked at the junction of the dermis and muscle layer (Fig. 1*a*). Six days after the injection, infiltration with lymphocytes, mononuclear cells, plasma cells, and macrophages was observed (Fig. 1*b*). A few giant cells with peripheral nuclei (resembling Langhaus giant cells) were also seen.

Delayed skin tests applied 85 to 90 days after sensitization were positive in 11 of 16 animals. Two other animals showed doubtful sensitivity, and three did not react to antigen. In another experiment, three animals exhibiting delayed sensitivity after receipt of antigen in complete adjuvant were subsequently injected four times (two intramuscular, one intradermal and one intracardial injection within 35 days) with 7 mg (total 28 mg) of antigen. Skin tests 30 days later were of the delayed type only.

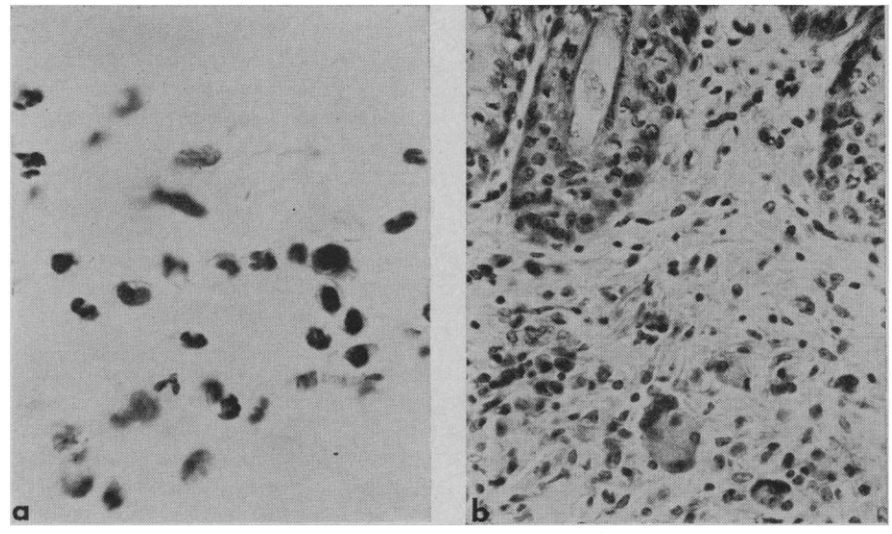

Fig. 1. *a*. Infiltration of polymorphonuclear neutrophiles and mononuclear cells on day after injection. *b*. Increased infiltration, chronic inflammatory cells, 6 days after injection.

Table 1. Delayed reactions in guinea pigs injected with *p*(Tyr, Glu)-*p*Ala--*p*Lys in complete Freund adjuvant (simultaneous multiple skin tests). Reactions are graded according to the scheme of Benacerraf and Gell (*13*). All tests were made on the 10th day except for two animals that received 2000 μg. These, tested on day 30, showed no response.

Positive animals (No.)	Delayed response in individual guinea pigs		
	Number of animals	Minimal response dose (μg)	Reaction to minimal dose
	4 animals; injection 500 μg		
0			
	14 animals; injection 1500 μg		
14	5	125	+
	2	125	++
	1	250	+
	2	250	++
	2	500	+
	1	500	++
	1	2000	+
	4 animals; injection, 2000 μg		
4	1	62	+
	1	125	+
	2	500	+
	2 animals; injection 3000 μg		
2	1	30	+
	1	250	+
	2 animals; injection 6000 μg		
2	1	30	+++
	1	30	+
	10 animals; injection 10,000 μg		
7	1	250	+
	1	500	±
	1	500	+
	1	1000	+
	3	1000	++

Animals sensitized with antigen in the complete Freund adjuvant were tested intradermally with a series of related copolymers. Positive delayed reactions were observed with *p*(Tyr,Glu,Ala), sample 44, and *p*(Tyr,Glu), sample 102. A doubtful reaction was observed with *p*(Tyr,Glu)--*p*Lys, sample 22; a negative reaction was observed with *p*Ala-*p*(Tyr,Glu)--*p*Lys, sample 28, and with *p*Ala-*p*(Tyr, Glu)--*p*(Lys,Ala), sample 30. These samples have been characterized previously (*4*) and their antigen specificity in rabbits has been studied (*10*). All these copolymers cross-reacted with rabbit antibodies to *p*(Tyr,Glu)-*p*Ala--*p*Lys both in in vitro tests (*10*) and in passive cutaneous anaphylaxis tests in guinea pigs (*11*) (Table 1).

Circulating antibodies to *p*(Tyr,Glu)-*p*Ala--*p*Lys in guinea pigs could be detected after a prolonged course of immunization (in 13 out of 25 animals after two intramuscular, one or two intradermal, and one intracardial injection within at least 110 days, tested 30 days later).

Relatively large amounts of antigen were used both as sensitizing and as skin-test doses in these studies. Furthermore, the intensity of the reactions was at a lower level than that which is readily induced by a number of other experimental systems. We conclude that a multichain synthetic polypeptide which has been shown to be antigenic in rabbits can be used to induce a 2-to-3 month period of delayed hypersensitivity in a majority of guinea pigs tested without eliciting the formation of detectable circulating antibodies (*12*).

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