formed together, as, for example, in birds' eggs, or marine shell-fish, the isotopic fractionation is found to be rather uniform at about 25 per mill (C^{13} enriched in the carbonate phase). Single-stage equilibrium fractionations for carbon compounds as large as 70 per mill at room temperature have been calculated by Urey (*12*). (In general, the direction of equilibrium fractionation effects is to concentrate C^{13} in phases of higher oxidation state relative to those of lower oxidation state.) Apparently mechanisms for the establishment of such equilibria at low temperatures are not found in nature on earth. Kinetic carbon isotope effects of the order of 40 or 50 per mill have been reported (*13*), but in the case of decarboxylation of organic acids, the direction of the effect is to concentrate C^{13} in the reduced carbon relative to the carbon dioxide. Multistage processes produce extreme isotope effects in terrestrial systems, such as the evaporation-precipitation cycles of water (*14*) in which a small single-stage fractionation of about 7 per mill for O^{18} can be repeated several times to give products which differ from the starting materials by as much as 40 per mill. Such multistage processes are unknown in the terrestrial carbon cycle; production of the isotopic anomaly in Orgueil would require processes in the meteorite parent body without terrestrial counterpart. Although the observed carbon isotope distribution in Orgueil is unlike any known terrestrial case, the possibility of its production by chemical means cannot be ruled out at the present time.

In the absence of biological activity, it may be very difficult to find chemical pathways connecting carbonate minerals and complex organic compounds at the low temperatures of meteorites. The isotopic differences observed may not be due to chemical isotope effects, but may represent an original inhomogeneous distribution of isotopes among chemical species resulting from a sampling of materials with different histories of nucleosynthesis. Among the most likely nuclides to show such effects should be C^{13}, N^{15}, and O^{17}, which were probably formed within the solar system by spallation reactions resulting from bombardment of small solid planetesimals by charged particles from the sun (*15*).

The other meteorite known to contain carbonates is Ivuna, which is also a carbonaceous chondrite. The only material available in these laboratories was a 1.3-mg hand-picked carbonate fraction, which produced 3.8 μmole of CO_2. This gas was compared with Orgueil sample A in the mass spectrometer, and was found to be indistinguishable from the Orgueil sample in isotopic abundances within a rather large error of about 1 percent in isotopic ratios (*16*).

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Passive Cutaneous Anaphylaxis with Antibody Fragments

Abstract. *Pepsin-digested rabbit antibody (5S) provoked reverse passive cutaneous anaphylaxis in the guinea pig, but was somewhat less effective, mole for mole, than the native antibody. Fixation of guinea pig complement by the pepsin-digested antibody could not be demonstrated either in vitro or in vivo. Splitting of the 5S fragment into two monovalent fragments (3.5S) markedly reduced the capacity to provoke reverse passive cutaneous anaphylaxis.*

Digestion with pepsin suppresses the reactivity of 7*S* rabbit antibodies with complement (C′) (*1*, *2*). The digestion yields 5*S* antibody fragments which precipitate and have two combining sites, like the undigested antibody molecules, but lack fragment III (*3*). Since it has been postulated that C′ plays an essential role in some forms of immediate hypersensitivity (*4*), 5*S* antibody fragments may prove useful in defining this role. Moreover, the 5*S* fragments can be split, by reduction of a single disulfide bond, into two 3.5*S* fragments, which are univalent and unable to precipitate (*5*). Previous reports indicated that the nonprecipitating "univalent") antibodies, which may be found in human and animal sera after immunization, can elicit passive anaphylaxis (*6*). Whether these undigested, nonprecipitating antibodies are indeed univalent is, however, uncertain (*7*). The univalence of the 3.5*S* fragments is more firmly established from quantitative hapten-binding studies (*3*). Comparison of the 5*S* and 3.5*S* antibody fragments may therefore help to assess the importance of antibody valence or molecular weight or both in immediate hypersensitivity.

Human gamma globulin (HuGG) was chosen as the antigen because it is one of the few (*8*) which is effective in the reverse as well as in the direct technique of passive cutaneous anaphylaxis (PCA) in the guinea pig, a well-established model of immediate hypersensitivity (*9*). To minimize the anticomplementary action of HuGG, fractional precipitation of unheated Cohn's Fraction II was performed with ammonium sulphate as described by Christian (*10*) and the supernatant of the 0.96*M* precipitation was used. With this preparation, C′ fixation of the antigen controls in the experiments to be described never exceeded five 50-percent hemolytic units ($C'H_{50}$) out of 100.

Antisera against HuGG were obtained by immunization of rabbits with HuGG and adjuvants. The gamma

globulin fractions of the antisera were precipitated with, and washed twice in, one-third saturated ammonium sulphate. Aliquots were digested with pepsin (1 mg enzyme per 100 mg substrate in acetate buffer, *p*H 4.0, ionic strength 0.1) and control aliquots were exposed to the same *p*H and temperature for the same length of time as the experimental preparations, but without pepsin. Both kinds of final preparations were dialyzed in the cold against an isotonic buffer, *p*H 7.3 (*11*). Quantitative determinations of C′ fixation and of precipitating antibody were made according to standard methods (*11*).

Although HuGG is known to fix C′ when aggregated by a number of agents (*12*), it failed to fix C′ when it was aggregated by pepsin-digested anti-HuGG. Quantities of HuGG ranging from 2 μg to 1.28 mg were mixed in optimal proportions with pepsin-digested antibody (10 μg to 6.4 mg of antibody protein), in presence of 100 units of guinea pig C′ and incubated at 4°C for 18 hours. No specific C′ fixation was detected. It is to be noted that the pepsin-digested antibody controls were not anticomplementary at all and that the HuGG antigen controls, as mentioned above, never fixed more than five units of C′. These results indicate that when HuGG and its pepsin digested specific antibody react, the complex does not fix C′, or only does so several thousand times less well than do systems comprising native antibodies (*13*).

Tests for PCA were performed as described (*9*). When pepsin-digested antibody was injected intradermally, followed by the intravenous injection of antigen (direct technique), no reactions were obtained, even when the pepsin-digested antibody was injected in amounts 1000 times greater than that sufficient for a definite reaction with the native antibody. Similar negative results with pepsin-digested antibodies were reported years ago in direct passive systemic anaphylaxis (*14*), and recently in direct PCA (*2*). In the present experiments, reactions failed to appear also when pepsin-digested anti-HuGG, injected intradermally, played the role of antigen, and sheep anti-rabbit gamma globulin was used as antibody (reverse technique). Thus, pepsin-digested antibodies are ineffective in the direct PCA system; their ineffectiveness may result from lack of sensitization of the skin by the pepsin-digested anti-HuGG. Furthermore, if

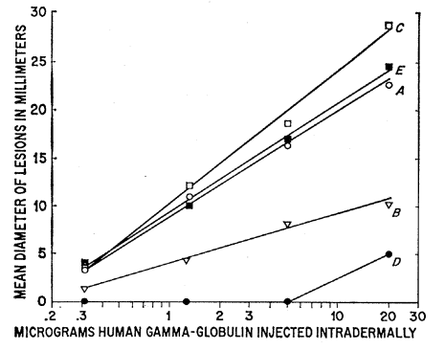

Fig. 1. Reverse passive cutaneous anaphylaxis with native (7*S*), pepsin digested (5*S*), and pepsin-digested and reduced (3.5*S*) rabbit antibody. Open circles, native anti-HuGG, 0.5 mg antibody protein; open triangles, pepsin-digested anti-HuGG, equimolar with open circle values; open squares, pepsin-digested anti-HuGG, same weight of protein as open circles; closed circles, same preparation as open squares after treatment with 0.02*M* mercaptoethanol; closed squares, same preparation as closed circles, amount increased 10 times.

the preparation of pepsin-digested anti-HuGG contained any residual undigested anti-HuGG, the latter was present in a concentration lower than one per thousand.

In reverse PCA the antigen is injected intradermally, followed, after a suitable interval, by antibody injected intravenously (*9*). Here the antigen sensitizes the skin and the antibody does not need the capacity to do so (*8*). Thus, elicitation of reverse PCA

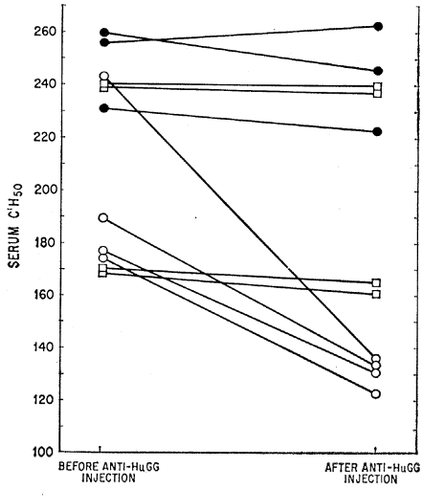

Fig. 2. Complement fixation in vivo by native and by pepsin-digested rabbit antibody. Closed circles, native anti-HuGG controls, 0.05 mg antibody protein (no antigen); open circles, native anti-HuGG, also 0.05 mg antibody protein; open squares, pepsin-digested anti-HuGG, same weight of protein as open circles.

by antibody can be assayed independently of skin sensitization (Fig. 1). Each of 30 guinea pigs, in five equal groups, was injected intradermally with 0.31, 1.25, 5 and 20 μg of HuGG. Five hours later, intravenous injections were given. One group received 0.5 mg of undigested anti-HuGG (antibody protein) and served as standard (group *A*). Two groups were injected with two different amounts of pepsin-digested anti-HuGG (5*S*) and two other groups with different amounts of pepsin-digested and reduced anti-HuGG (3.5*S*). The native preparation was more effective than an equimolar preparation of pepsin-digested antibody, whose protein content had decreased by 32 percent (group *B*). However, when the protein content of the pepsin-digested preparation was equal to that of the native antibody, the reactions were equivalent (group *C*). The fact that pepsin-digested antibody gave smaller size skin reactions when compared with equimolar concentrations of the native antibody needs further explication. With very small reactions (10 mm in diameter or less) it is very difficult to obtain precise data and therefore valid comparison cannot be made. The most valid comparison may be obtained by provoking significant reactions of comparable size with different preparations of antibody and maintaining constant the intradermally injected antigen. An increase of about 50 percent (equal weight of protein) of the pepsin-digested antibody gave approximately the same reaction as the native antibody. Reduction with 0.02*M* mercaptoethanol decreased the reactivity of this antibody preparation to an insignificant level (group *D*), although residual reactivity could be demonstrated with a tenfold increase in the amount of reduced antibody fragment (group *E*).

It is conceivable that the fixation of complement is dependent on different factors in vivo and in vitro; specifically, living tissues, to which either the antigen or the antibody may become bound, could endow the antigen-antibody complex with C′-fixing ability, despite the absence of fragment III from the antibody molecule. To test this possibility, C′ was determined in the serum of guinea pigs before and after reverse PCA (Fig. 2).

Each of eight guinea pigs was injected intradermally with HuGG and three guinea pigs were injected with equal volumes of diluent. Two hours

later blood samples were obtained by intracardiac puncture and C′ was determined (left-hand values). Five hours after the intradermal injections, four animals of the first group were injected intravenously with 0.5 mg pepsin-digested anti-HuGG and the remaining four, as well as the three previously injected with diluent, with 0.5 mg native anti-HuGG. A second sample of blood was obtained 10 minutes after the intravenous injection and C′ was redetermined (right-hand values). The serum from animals injected intradermally with diluent showed only slight changes after injection with native antibodies (negative control). Those sera from animals injected intradermally with HuGG and intravenously with native anti-HuGG showed marked drops (positive control). The sera from the experimental group, injected intradermally with HuGG and intravenously with pepsin-digested anti-HuGG showed only insignificant changes.

The experiments reported indicate that equivalent weights of pepsin-digested and native antibodies have equal capacities to produce the reverse PCA phenomenon in the guinea pig. In contrast, these studies do not demonstrate in vitro or in vivo C′ fixation by the pepsin-digested anti-HuGG-HuGG system. Splitting bivalent 5*S* antibody fragments into 3.5*S* monovalent fragments sharply decreases reverse PCA activity. Hence, the valence or the molecular weight of an antibody molecule or both appear to be of critical importance in the mechanism of the reverse passive cutaneous anaphylaxis reaction (*14*). Since this paper was submitted, a paper has been published which questions the role of naturally occurring nonprecipitating or "univalent" antibodies in immediate hypersensitivity (*15; 16*).

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Lingual Vein Injection in the Rat

Abstract. *The difficulties encountered with repeated intravenous injections in rodents limit many experiments. A method is described which employs the lingual veins of anesthetized rats and which allows for repeated intravenous administration of fluids and cells in young rats and hamsters.*

The difficulties of surgery on small animals limit many experiments. In particular, the low number of repeated intravenous injections that can be given to small laboratory animals is a common restriction forced upon experimentalists by the fragility and paucity of superficial veins. The advantages of making injections into the lingual veins of the rat have been demonstrated and the technique is now described, since it is neither used commonly nor presented in standard works (*1*).

The anesthetized rat is placed on its back and the tongue is drawn out with fine-toothed forceps to one side of the incisor teeth. The body of the tongue is wiped dry with gauze and held between the left thumb and forefinger. Traction is applied without hindrance to respiration, so that the pair of veins on the under surface are exposed near the root of the tongue. If open ether anesthesia is used, it can be maintained by inserting the upper jaw and nostrils of the rat into a narrow glass or metal anesthesia cone; at the same time the injection is made easier by the stabilization of the head and neck. A sharp, 25-gauge needle easily enters even the small lingual veins of young 50- or 60-g rats. The point of the needle and the entering injection fluid can be seen readily through the thin wall of the vein. With the animal suitably placed on a small operating board and the operator's hands comfortable, the position can be held for 15 to 20 minutes while large volumes or slow infusions are given. Finger and thumb pressure is applied over the puncture as the needle is withdrawn and for 2 or 3 minutes thereafter. This prevents the formation of a perivenous hematoma which would jeopardize repeated venipunctures.

In one series of experiments, courses of intravenous injections were given to 45 rats by the lingual route. At least six injections were given to each rat on alternate days and into alternate lingual veins. All the injections were successful. The method is applicable to hamsters and to other small animals used in the laboratory (*2*).

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Reaction Time as a Function of the Cardiac Cycle in Young Adults

Abstract. *Simple reaction times to auditory stimuli varied with the phase of the cardiac cycle in which the stimuli were presented, tending to be fastest to stimuli presented during the P-phase of the electrocardiogram. One hundred reaction times obtained from each of 56 men and women between the ages of 20 and 30 years were analyzed.*

Reaction time in human subjects is related to certain aspects of cardiovascular functioning, pulse rate (*1*), variation in pulse rate (*2*), and blood pressure (*3*). Also, earlier work has shown a relationship between reaction time and the electroencephalogram (EEG) (*4*). Arterial pressure fluctuations, by affecting the baroreceptors of