47; therefore, the cosmic-ray exposure age, t, is between 7000 and 25,000 years.

If the measurements of rare-gas isotope by Kirsten *et al.* (*4*) are correct, the discrepancy between the content of rare-gas isotope and radioactive isotope implies that the Farmington meteorite might have been shielded by approximately 3 m of material until 25,000 years ago when a collision exposed the material. Three meters of shielding reduces the isotope production by a factor between a hundred and a thousand. If the material is in this shielded state for many million years, the Al^{26} radioactivity only builds up to saturation which is between 0.05 and 0.5 disintegrations per minute per kilogram. However, for the stable isotopes there is no saturation, and He^3, Ne^{21}, and Ar^{38} continually build up with time. The amounts of the stable isotopes produced during the many million-year period can exceed the amounts produced during the last 25,000 years. If the measurement of the Ar^{38}/Ne^{21} ratio of Kirsten *et al.* is correct, it would indicate that Ar^{38} production is greatly enhanced in relation to Ne^{21} production under 3 m of shielding by the action of secondary neutrons on calcium (*9*).

J. DEFELICE
G. G. FAZIO
E. L. FIREMAN
Smithsonian Astrophysical Observatory, Cambridge, Massachusetts

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9 September 1963

Binding Capacity of Reductively Fragmented Antibodies to the 2,4-Dinitrophenyl Group

Abstract. *Mild reductive cleavage of the polypeptide chains of antibodies to the 2,4-dinitrophenyl group has been carried out according to the methods of Porter and co-workers. The addition of ε-2,4-dinitrophenyllysine permitted almost complete retention of the antibody binding capacity upon transfer to 1*M *propionic acid. Fractionation of the chains showed that the hapten was specifically bound to the A and not the B chains, and that about 30 percent of the B chains were bound to A in the presence of the bound hapten. The results confirm the conclusion of Porter and co-workers that the A chain is the principal component of the antibody site, but suggest that the B chain may also contribute to the site.*

It is generally accepted that a 6.6*S* γ-globulin molecule consists of two principal types of polypeptide chains linked by disulfide bridges (*1, 2*). These two types are designated H and L by one group of investigators (*2*), and A and B by another (*3*). We will use the A and B designations. The question arises concerning the relationship of these chains to the specific activity of 6.6*S* γ-globulin antibody molecules. Because discrete bands of the B chains, but not of the A chains, of pure antibodies were produced by electrophoresis in starch gels in urea, and because the nature of the band patterns varied with the specificity of the antibodies. Edelman *et al.* (*4*) inferred that the B chains were probably associated with antibody activity. Under the conditions they used to prepare them, however, the separated A and B chains were both inactive. Fleischman *et al.* (*3, 5*), working with γ-globulin preparations containing antiprotein antibodies, separated the A and B chains by methods sufficiently mild to permit the retention of a substantial part of the original antibody activity in certain systems. While neither the A nor B chains retained the capacity to precipitate with the specific protein antigen, the A chain and not the B would coprecipitate with the specific antigen-antibody precipitate. Antibody activity is difficult to measure quantitatively by this method. It is desirable to perform related experiments with pure anti-hapten antibodies whose specific activity can be unambiguously measured. Such experiments have been performed by Utsumi and Karush (*6*), and also in our laboratories.

In our experiments, we used purified rabbit antibodies to the 2,4-dinitrophenyl determinant (anti-DNP) (*7*). These antibodies are characterized by an unusually large affinity for the homologous hapten ε*N*-2,4-dinitrophenyllysine (DNP lysine) (*8*); this was considered advantageous for the retention of antibody activity under adverse conditions. The inter-chain disulfide bridges of anti-DNP and normal rabbit γ-globulin were reduced with 0.2*M* mercaptoethanol in 0.55*M* tris buffer at *p*H 8.2 and the free sulfhydryl groups were alkylated with iodoacetamide exactly according to the procedure of Fleischman *et al.* (*3, 5*). In each of two sets of experiments a portion of anti-DNP and of normal γ-globulin were treated in parallel. In the first set, after dialysis overnight against 0.16*M* NaCl, the reduced, alkylated proteins were dialyzed against 1*M* propionic acid, and 50 mg of each protein was applied successively to a column 75 × 2.8 cm of Sephadex G-75 equilibrated with 1*M* propionic acid. The results of the fractionation are shown in Fig. 1*A*. Absolute yields were, within experimental error, 100 percent of the protein applied. In these, and a substantial number of similar experiments, the relative yields under peaks A and B were 75 ± 1 and 25 ± 1 percent, respectively, entirely consistent with the observations of Fleischman *et al.* (*3*).

In the second set of experiments, the anti-DNP and normal γ-globulin samples were also reduced, alkylated, and dialyzed against 0.16*M* NaCl. However, at this point, the dialysis bags were opened; 2 moles of DNP lysine per mole protein were added to each bag, and the solutions were then dialyzed against 1*M* propionic acid containing $6 \times 10^{-6}M$ DNP lysine. By thus adding the specific hapten to the reduced anti-DNP before exposing the protein to 1*M* propionic acid, appreciable binding capacity could be retained upon subsequent exposure to the acid. From absorbance measurements made at 280 and 360 mμ against the dialyzate as a blank, with the extinction coefficient, $E_{1cm}^{1\%} = 14.6$ at 280 mμ for the protein and $E_{360m\mu} = 0.89 \times 10^4$ for antibody-bound DNP lysine (*9*), r, the number of moles of DNP lysine bound per mole of antibody protein, was calculated to be 1.65 at a protein concentration of 0.93 mg/ml in the 1*M* propionic acid containing $6 \times 10^{-6}M$ DNP lysine. The normal γ-globulin sample, on the other hand, after correction for the small protein absorbance at 360 mμ, had the same absorbance at 360 mμ as the dialyzate, and there-

fore had bound no DNP lysine. To achieve comparable conditions for the subsequent chromatographic experiments, 1.5 moles DNP lysine per mole of protein were therefore added to the normal γ-globulin sample. Both protein samples were then successively applied to the Sephadex G-75 column equilibrated with 1*M* propionic acid containing $6 \times 10^{-6}M$ DNP lysine. The elution patterns from absorbance measurements at 280 and 360 mμ with the equilibrating mixture as blank are shown in Figs. 1*B* and 1*C*.

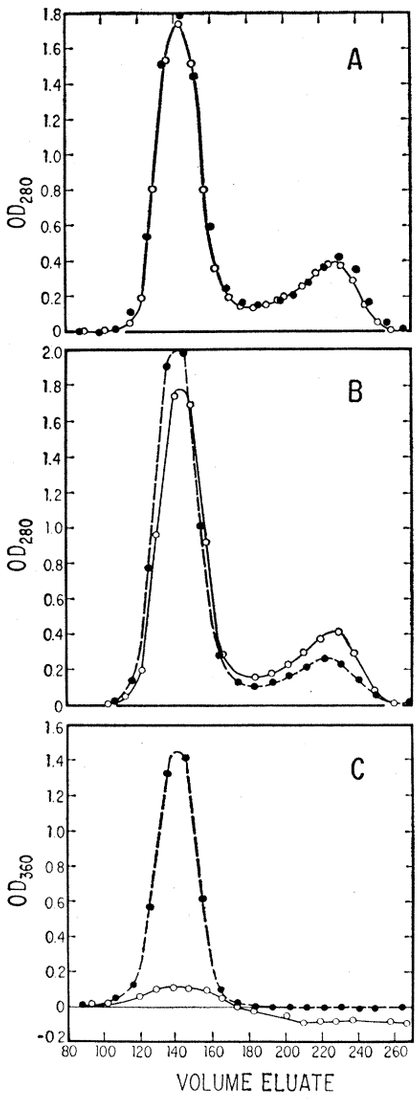

Fig. 1. (*A*) Protein elution patterns of reduced, alkylated normal rabbit γ-globulin (open circles) and anti-DNP antibodies (closed circles) from the same G-75 Sephadex column equilibrated with 1*M* propionic acid; (*B*) protein elution patterns; (*C*) DNP lysine elution patterns, of reduced, alkylated normal rabbit γ-globulin (open circles, solid line) and anti-DNP antibodies (closed circles, dashed line) containing excess DNP lysine (see text), from a G-75 Sephadex column equilibrated with 1*M* propionic acid containing $6 \times 10^{-6}M$ DNP lysine.

The figures show (i) DNP lysine was associated with the A peak of the reduced alkylated anti-DNP, and not significantly with the B peak, under conditions where essentially no association of DNP lysine with either the A or B peaks of normal γ-globulin occurred. The integrated absorbance at 360 mμ under the A peak, corrected for protein absorption, corresponds to an *r* value of 1.35 referred to the original antibody molecule (that is, to the total protein). This amounts to 82 percent of the bound hapten applied to the column. Since anti-DNP and DNP lysine are in equilibrium, one might expect some bound DNP lysine to be released from the protein during chromatographic separation, and to migrate more slowly on the Sephadex; hence the true recovery of binding capacity under the A peak might have been even greater than 82 percent of the original. (ii) There was a distinct transfer of protein from the B to the A peaks in the sample of anti-DNP to which the DNP lysine was specifically bound. The relative amounts of protein under the A and B peaks were 84 and 16 percent, respectively, and the absolute recovery was 101 percent. The contribution of the excess DNP lysine to the absorbance of the protein solution at 280 mμ was negligible. In a similar experiment carried out several months later with a different batch of anti-DNP on a freshly prepared column, the same distribution (84 to 16) of protein was obtained. This change in distribution from 75 : 25 to 84 : 16 is, in our experience, entirely outside of experimental error. Furthermore, this is a specific effect, since normal γ-globulin fractionated on the same column (Fig. 1*B*), equilibrated with $6 \times 10^{-6}M$ DNP lysine and 1*M* propionic acid, showed a typical 76 to 24 percent distribution between the A and B peaks.

From these results it is apparent that the anti-DNP antibody activity is primarily associated with the A chains (*3, 5*). Although some B chains of anti-DNP apparently formed complexes with A in the presence of the hapten, the hapten-binding capacity associated with the A peak cannot be attributed exclusively to these complexes. Thus, while 82 percent or more of the hapten-binding capacity was recovered in the A peak, only about 30 percent of the original amount of B chains were bound to A chains (Fig. 1*B*). Furthermore, the B chains which were free did not measurably bind the hapten. These results therefore confirm the conclusions reached by Fleischman *et al.* (*3, 5*), as do the results of Utsumi and Karush (*6*), that the A chain is of primary importance in defining the antibody site.

The specific association of A and B chains produced by the hapten may be explained by the hypothesis that, on binding the hapten, the A chains of anti-DNP are maintained in a conformation more nearly native than in the absence of the hapten in 1*M* propionic acid, and in this conformation, A and B associate. This hypothesis accords the B chains no direct participation in the antibody site. On the other hand, the results obtained may also be explained if the B chains do indeed participate directly, if only in a secondary way to the A chains, in the formation of the antibody sites. For example, a B chain may contribute one or a few amino acid residues to an antibody site in such a position that their removal only somewhat diminishes, but does not destroy, the binding affinity of the site. This possibility is supported by the observations, (*10*), that the specific labeling of the active sites of anti-DNP antibodies with *p*-nitrophenyldiazonium fluoborate (*11*), and of anti-benzenearsonate antibodies with *p*-benzenearsonate diazonium fluoborate (*12*), results in the formation of azotyrosine groups of both the A and B chains of the two kinds of antibodies. The labeling results are interpreted (*10*) to mean that a tyrosine residue of the B chain is either part of, or very close to, the active sites of both kinds of antibodies. Furthermore, differential labeling with iodine isotopes of the active sites of anti-benzenearsonate antibodies (*13*) also suggests that a tyrosine residue on the B chain is part of the antibody sites. Therefore, while there is good evidence that the A chain plays the major role in providing the structure of the antibody site, the B chain may also contribute to it (*14; 15*).

H. METZGER
S. J. SINGER
Department of Biology, University of California, La Jolla

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15. Supported by grant E-4246 from the U.S. Public Health Service. We thank Professor R. R. Porter for acquainting us with methods and results from his laboratories. One of us (H.M.) is a Helen Hay Whitney Foundation fellow.

20 August 1963

Quartan-Type Malaria Parasite of New World Monkeys Transmissible to Man

The first recognized natural transmission of a simian malaria to man occurred accidentally in 1960 with the vivax-like *Plasmodium cynomolgi bastianellii* as reported by Eyles, Coatney and Getz (*1*). In 1961 Coatney *et al.* (*2*) and Schmidt *et al.* (*3*) showed that an old laboratory strain of *P. cynomolgi* could also be transmitted to man by mosquito bite.

Recently we have been able to transmit to a man a second species of simian malaria, *Plasmodium brasilianum*. This was accomplished by the bites of infected *Anopheles freeborni* mosquitoes which had been allowed to feed on a spider monkey, *Ateles geoffroyi geoffroyi*, with an infection acquired naturally in Panama. This quartan parasite of New World monkeys was originally described by Gonder and von Berenberg-Gossler (*4*) from the white or bald oukari, *Brachyurus calvus*. Clark (*5*) and Clark and Dunn (*6*) reported it as a natural parasite of *Ateles sp.*, *Cebus sp.*, and *Alouatta sp.* Clark and Dunn were unable to infect man with this parasite either by the inoculation of parasitized blood from the monkey or by the bites of infected *Anopheles albimanus* mosquitoes.

Our transmission of *Plasmodium brasilianum* to man by mosquito bite was possible because nine individuals (seven Caucasians and two Negroes) volunteered to participate. They were bitten by 8 to 15 infected mosquitoes. Five of the volunteers (three Caucasians and two Negroes) developed patent infections. The prepatent periods ranged from 29 to 64 days, with a mean and a median of 43 days. The parasitemias were of low order, less than 50 parasites per microliter of blood, and the durations of patent parasitemia ranged from 4 to 19 days, during which time gametocytes were occasionally observed. Fever was present in only one volunteer who exhibited a true quartan fever pattern with a maximum temperature of 39.5°C. This same volunteer experienced a fever of 38.3°C 2 days preceding the onset of patent parasitemia. Symptomatology was minimal, consisting only of headache and loss of appetite.

The infection in man has been blood-passaged back to the monkey and to additional human volunteers. Thus the identity and infectivity of the parasite have been confirmed.

It is of interest to note that *Plasmodium brasilianum*, the quartan simian parasite of New World monkeys was transmitted with ease to both Caucasians and Negroes, whereas the vivax-like parasite of Old World monkeys, *P. cynomolgi* and *P. cynomolgi bastianellii*, was transmissible only to Caucasians (*2, 7*). This finding parallels the situation as found with *P. malariae* and *P. vivax* in man.

The fact that humans can be experimentally infected by the bites of mosquitoes infected with *P. brasilianum* constitutes a second example of a zoonotic malaria. This situation is of special interest because of the possible importance of these zoonoses to worldwide eradication.

PETER G. CONTACOS*
JOSEPH S. LUNN*
G. ROBERT COATNEY
JOHN W. KILPATRICK†
FRANCES E. JONES†
Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, Bethesda 14, Maryland

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* Present addresses: Malaria Project, U.S. Penitentiary, Atlanta, Ga.; † P.O. Box 195, Chamblee, Ga.

22 August 1963

Hepatomas in Rainbow Trout: Descriptive and Experimental Epidemiology

Abstract. *Four ingredients of a dry diet, Santa Monica, and a special food supplement, were tested for their effects on the occurrence of hepatomas in* Salmo gairdnerii. *When cottonseed meal was omitted from the diet, no hepatomas developed in the experimental fish. When the same diet with its usual cottonseed meal component was fed, 48 percent of the fish developed hepatomas.*

Hepatomas were reported among rainbow trout (*Salmo gairdnerii*) by Haddow and Blake (*1*) in the British Isles, Cudkowicz and Scolari (*2*) in Italy, and Nigrelli (*3*), Wales (*4*), and Ellis (*5*) in the United States, prior to 1960. The discovery of hepatomas in hatchery-reared rainbow trout in 1960 was described by Rucker *et al.* (*6*); this outbreak, which proved to be nationwide in distribution, was exceptionally high in incidence among some groups of trout. Wood and Larson (*7*) mention a 50 percent occurrence of gross tumors among 250,000 adult rainbow trout.

Hepatoma lesions vary greatly among rainbow trout. Their histopathology has been studied and described by Rucker *et al.* (*6*), Wood and Larson (*7*), and others (*2, 8*). Metastases have been reported in the kidney and spleen (*6–8*), and the heart, stomach, and pyloric cecae (*2*).

In 1960, after the initial discovery of hepatoma in California in a shipment of Idaho trout and, subsequently, among dry-fed fish at a number of State hatcheries, we decided to survey the entire fish population in State hatch-