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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.

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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.

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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-

eries throughout California and obtain information on the epidemiology of this disease. The results of this survey, as shown in Table 1, indicated that trout raised on meat (liver, lung, spleen) and fish (marine rockfish) were free of hepatomas, whereas those raised on dry diets (Stockton and Santa Monica) had the disease in varying degrees. This association between diet and the occurrence of hepatomas appeared to be independent of the general environment. Trout of equal weight from the Hot Creek hatchery showed no hepatomas when fed the Stockton or the meat and fish diets, but seven out of 14 in the group fed on the Santa Monica diet had hepatomas. Instead, it appeared that diet was the critical factor in tumor induction, so we turned the investigation to the details of the dietary composition.

The dry feeds consisted of complex mixtures of animal and vegetable meal, vitamins, minerals, antioxidants, antibiotics, antifungicides and other substances. The Santa Monica diet, for example, contained 44 separate ingredients. A helpful clue in reducing the number of suspect ingredients came from the "Summary of Trout Hepatoma Survey" (*9*), in which it was reported that hepatomas developed in fish after giving them rations similar to a Cortland-6 diet. This ration consisted of about 50 percent beef liver and pork spleen, 2 percent salt, plus 12 percent each of fish meal, cottonseed meal, distillers solubles, and wheat middlings. As these last four ingredients were also contained in the dry rations, Stockton and Santa Monica, our feeding experiments were designed to test their effects as well as the effect of a special supplement in the Santa Monica ration.

The experiments were conducted at two hatcheries belonging to the California Department of Fish and Game: San Joaquin, where the temperature of the water ranged from 6.7°C to 10°C; and Darrah Springs, where the temperature ranged from 14.5°C to 15.5°C. All fish used in the diet trials were hatched from one day's spawntaking operation at the Mt. Shasta Fish Hatchery. The parent fish were fall-spawning rainbow trout (*Salmo gairdnerii*). The eggs were divided into groups, each of which would provide approximately 20,000 first-feeding fish. Each group was placed in a separate pond and the groups within each hatchery received water identical in quality, temperature, volume, and rate of flow. Throughout the entire experiment the trout appeared in good health, insofar as they were free from the diseases which sometimes affect hatchery fish. Their growth was normal within each hatchery.

Except for the special diets, these trout received the standard care of other hatchery fish. All diets were fed according to a schedule in which the amount and size of food pellets were prescribed according to size of fish and the water temperature. As the diet trials progressed and the fish grew, their numbers were reduced by seining up each separate group and removing a predetermined weight of fish based on growth and weight records. This thinning process was carried out among all groups of fish at approximately the same time, and each lot was reduced in weight so that the remaining weight of fish, and their approximate number, were the same in each group.

The ingredients of each diet, including the control diet, were obtained from the same suppliers throughout the experiments. The complete Santa Monica formula was used as the control diet; the ingredients of this diet included: 19 percent cottonseed meal (41 percent protein, solvent extracted, degossypolized); 3 percent distillers solubles; 25 percent wheat middlings; 19 percent fish meal; and a 3 percent special supplement containing antioxidant. These ingredients were all suspect, and were tested either by omission from the dry control diet, or by addition to beef liver, as shown in Table 2. The beef liver was ground and mixed with the dry ingredients prior to feeding.

The fish fed on diet 8 were examined by one of us (H.W.), but the livers of the fish fed on all the other diets were submitted to an independent pathology laboratory for examination. After 23 months, 48 percent of the control group at San Joaquin had developed tumors, whereas 80 percent of the controls at Darrah Springs developed tumors after only 10 months. This difference is believed to be due to the higher water temperature at Darrah Springs. As the water temperature increases, the food intake also increases, together with the metabolic and growth rates; these, in turn, affect the rate of tumor induction.

The most significant observation was that the fish fed on diet 2, Santa Monica, minus cottonseed meal, developed neither gross nor microscopic hepatomas during the course of the experiment. In contrast, 33 to 50 percent of the fish fed on the Santa Monica diet 1, and its other modifications (diets 3, 4, and 5), had developed tumors after 23 months. This indicates that the cottonseed meal was necessary for the development of hepatoma and suggests that a carcinogen was present. Whether the agent is extrinsic or intrinsic to the cottonseed meal is not known and

Table 1. The occurrence of hepatomas in rainbow trout in hatcheries in California, 1960.

Hatchery	No. of fish examined	No. with hepatomas	Approximate No. of fish per kg
Stockton (dry feed)			
Moccasin Creek	27	1	11–23
Hot Creek	7	0	21
Kern	17	0	13
Darrah Springs	49	0	13
Mt. Shasta	17	1	11
Mojave River	16	0	11
San Joaquin	14	0	9
Santa Monica (dry feed)			
Moccasin Creek	7	2	23
Hot Creek	14	7	21
Fish Springs	7	5	17
Meat and fish			
Hot Creek	7	0	21
Mojave River	7	0	15
Hot Creek	6	0	†
Mt. Whitney	6	0	‡

* Revealed by microscopy. † Broodstock which measured approximately 34 cm. ‡ Broodstock which measured 22 to 45 cm.

Table 2. The effects of experimental diets on the occurrence of hepatomas in rainbow trout in California, 1961–63. All diets were fed for 23 months, except diet 8, which was fed for only 10 months. (S.M., Santa Monica)

Diet	No. of fish examined	No. with hepatomas*
San Joaquin hatchery		
1 S.M. (control)	50	24
2 S.M., no cottonseed meal	37	0
3 S.M., no distillers solubles	28	13
4 S.M., no wheat middlings †	20	10
5 S.M., no supplement	33	11
6 Liver (85%) plus wheat middlings (15%)	62	4
7 Liver (85%) plus fish meal (15%)	28	0
Darrah Springs		
8 S.M. (control)	50‡	40
9 Liver (85%) plus cottonseed meal (15%)	65	3
10 Liver (85%) plus distillers solubles (15%)	13	0

* Revealed by microscopy. † 4.5 kegs (10 lb) of wheat starch added for each 11.3 kegs (25 lb) of wheat middlings removed. ‡ 40 gross tumors confirmed microscopically, remaining fish not sectioned.

awaits definitive biochemical and biological studies.

The control diet at Darrah Springs, diet 8, contained 19 percent cottonseed meal. In contrast, diet 9 (liver and cottonseed meal) contained 37 percent cottonseed meal when calculated on a dry-weight basis. Yet at the end of 10 months, 40 out of 50 trout in the group fed on diet 8 had gross hepatomas, but neither gross nor microscopic tumors were found in the 50 trout sampled from the group fed on diet 9. At the end of 23 months, among 65 fish fed on diet 9 only 3 gross tumors were found. These observations suggest that liver markedly inhibits the hepatoma-inducing effect of the cottonseed meal, but does not completely suppress it. Nakahara *et al.* (*10*) have demonstrated the inhibitive effect of a diet containing liver on hepatoma induction by butter yellow (dimethyl-amino-azobenzene) in rats, and a similar effect may be operating in this instance.

The results obtained with diets 2 and 4 obscure the significance of the four microscopic tumors found in diet 6. With the omission of wheat middlings in diet 4, the incidence of hepatomas was no lower than in the control group; with diet 2, no hepatomas were found, although the diet included 25 percent wheat middlings. For these reasons, we believe that this component played no significant role in the outbreak of hepatomas in California during 1960, when the incidence of tumors in very young fish was extremely high (*11*).

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Antigens and Enzymes Made Insoluble by Entrapping Them into Lattices of Synthetic Polymers

Abstract. *Biologically active, insoluble products have been prepared from soluble antigens or enzymes by entrapping them into the lattice of an insoluble, highly cross-linked synthetic polymer as it forms. Antigens, made insoluble, removed quantitatively certain or all antibiotics from complex mixtures. Seven enzymes so treated are described.*

Soluble antigens have previously been rendered insoluble either by coupling the proteins with diazotized cellulose derivatives (*1*) or by linking them to the acid chloride of a carboxylated ion-exchange resin (*2*). Also, insoluble forms of amylase, pepsin, carboxypeptidase, and ribonuclease have been obtained by coupling with diazotized polyaminostyrene (*3*), and insoluble ribonuclease was obtained by absorption on a Dowex-50 cation-exchange resin (*4*). Tryspin (*5*) and papain (*6*) were transformed into insoluble derivatives by the introduction of polytyrosyl peptide side chains. This reaction is not applicable without considerable modifications to other proteins, or protein mixtures. Insoluble proteins obtained by coupling with diazotized polymers or by attachment to ion-exchange resins either suffered partial denaturation during these reactions or exhibited partial reversibility of the absorption, under certain conditions (*5*).

We have obtained biologically active, insoluble forms of antigens, enzymes, and other macromolecular material (for example, amylopectin), by mechanically entrapping the soluble macromolecular product into the lattice of a highly cross-linked synthetic polymer by polymerizing certain synthetic monomers in aqueous solution in the presence of the biologically active macromolecular substance to be embedded. Cross-linked polyacrylamide, which polymerizes in an aqueous medium and which has been used as a supporting medium for zone electrophoresis (*7*), is eminently suited for this purpose. We have used two methods for rendering proteins or other macromolecular biologically active material insoluble, depending on the purpose for which the resulting products are to be used. For rendering proteins or other antigens insoluble (procedure I) 50 mg of antigen are added to 200 mg of acrylamide, 120 mg of *N,N'* methylenebisacrylamide, 5.6 mg of tetramethylethylenediamine, 0.5 mg of Al $NH_4(SO_4)_2 \cdot 12\ H_2O$, and 1 ml of (1*M*) tris buffer, *p*H 8.6, all diluted to 11 ml with water. At the last, 2.5 mg of potassium persulfate are added. For rendering enzymes insoluble (procedure II), 2 mg of the enzyme are added to a mixture similar to the one described for procedure I except that the acrylamide is omitted and the amount of the potassium persulfate added is 5 mg.

In both procedures, the mixture is kept for 1 hour at 35°C without agitation after the polymerization catalyst (potassium persulfate) has been added. The insoluble synthetic polymer thus formed is dispersed mechanically, centrifuged for 30 minutes in a Sorvall centrifuge, model RC-2, rotor SS-34, at 17,000 rev/min (34,000*g*), and is washed by mixing it with 20 ml water and subsequent centrifugation. A total of three washings for procedure I and 16 washings for procedure II are necessary.

Table 1. Activity of crystalline enzyme (*9*) entrapped in the synthetic polymer preparation and the residual activity remaining in solution after the removal of the synthetic polymer. The "entrapped" activity is the percentage of total amount of enzyme present during the polymerization reaction. The "residual" activity is measured by first incubating the substrate at 25° to 35°C with insoluble enzyme for periods ranging from 25 to 180 minutes, cooling the mixture to 0°C, and removing the insoluble enzyme by centrifugation and subsequent filtration, and incubating the remaining solution at 25° to 35°C for 18 to 22 hours. Residual enzyme activity is expressed in percent activity per unit of time before centrifugation.

Enzyme	Activity	
	Entrapped (%)	Residual in supernatant solution (%)
Trypsin *	4–5.5 †	0.65
α-Chymotrypsin *	4.5	0.77
Papain *	3.4–6 †	0.57
α-Amylase ‡ §	1.9	0.7
β-Amylase || §	6.55	0.3
Ribonuclease ¶	4.6	0.89
Aldolase * *	4.2	0.52

* Determined by the casein digestion method (*10*). † Two separate experiments. ‡ From hog pancreas. § Determined by the reductometric method with dinitrosalicylic acid reagent (*11*). || From sweet potatoes. ¶ From beef pancreas; activity determined by the spectrophotometric method (*12, 13*). ** From rabbit muscle; activity determined by the method of Taylor *et al.* (*14*).