ponents is not as yet answered.

It should be emphasized that the 19S γ globulin preparations analyzed in this study were never obtained 100% pure. Low molecular weight proteins could be almost completely removed and they presented a relatively minor problem. However the components with a sedimentation rate greater than 19S could never be eliminated entirely. The possibility remains that these materials may not be solely aggregates of the 19S fraction and cause slight alterations in the measured carbohydrate-protein ratio.

In the human, the blood group antibodies, certain abnormal red cell antibodies, and part of the Wasserman antibodies have been thought to be localized in the 19 S fraction of γ globulin. The possibility is raised that these and perhaps other antibodies are rich in carbohydrate. The increased carbohydrate, particularly sialic acid, would influence such properties as the isoelectric point and solubility and might confer unique characteristics to such antibodies.

The absence of detectable 19 S components of the γ_1-globulin type in the sera of 2 patients with agammaglobulinemia was reported previously(13). Further observations have confirmed these findings although small amounts were found in one case where some 7 S material was also present. These observations offer further evidence for a possible relationship between the heavy components under discussion and antibodies.

Summary. 1. The heavy components (19 S) have been highly concentrated from normal human serum by repeated cycling in the preparative ultracentrifuge. Electrophoresis of such preparations has permitted isolation of a high molecular weight γ-globulin fraction. 2. Analyses of this material indicate a high carbohydrate content with hexose sugars and sialic acid present at approximately 5 times the concentration found for the main 7 S γ-globulin. The hexosamine-hexose ratio is approximately 0.5. 3. Markedly reduced levels were found in the sera of 4 patients with agammaglobulinemia. The possibility that certain antibodies fall into the 19 S fraction and are similarly rich in carbohydrate is discussed.

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Oxidation of Acetate and Malonate in Extrahepatic Tissues.* (22691)

ARNE N. WICK, JACK B. WOLFE AND HENRY I. NAKADA.
Scripps Clinic and Research Foundation, La Jolla, Calif.

Previous studies from this laboratory have shown that a rapid "explosive-like" oxidation of acetate occurs in the extrahepatic tissues (1). To investigate the pathway of terminal oxidation of acetate, the ability of C^{14} acetate to be oxidized in the presence of malonate, a competitive inhibitor of succinic dehydrogenase, was tested. This procedure, which has been successfully applied to *in vitro* studies (2,3) and which has been reported effective in whole animal experiments(4,5), proved to be non-inhibitory to acetate oxidation at concentrations of malonate that were not lethal to the eviscerated animals. Radioactive mal-

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TABLE I. Effect of Single Injection of Malonate on Oxidation of Radioactive Acetate. Results expressed as counts recovered in each period.

Period (30 min.)	Malonic acid administered 200 mg/kilo Exp. 511*	250 mg/kilo Exp. 474*
1	4364	3350
2	5391	3350
3	3293	2925
4	5031	2925
5	4563	3020
6	4648	2640
Avg	4550	3035
7	3219	3120
8	4421	2860
9	4632	2500
10	5263	2600
11	4519	2800
12	5249	3140
Avg	4560	2837

* In Exp. 511 the acetate was inj. at rate of 100 mg/kilo/hr calculated as acetic acid. In Exp. 474 tracer doses of acetate were administered.

onate was therefore utilized to investigate this apparent non-inhibitory effect of malonate on acetate oxidation. The results obtained are reported here.

Methods. General treatment of animals. Non-fasted rabbits were eviscerated-nephrectomized(6) and maintained at normal blood sugar at all times. A tracheal cannula was inserted at the time of operation to collect the expired CO_2 for C^{14} determinations. *Procedure used in determining the effect of malonate on acetate oxidation* (*Table I*). Carboxyl-labeled C^{14}-acetate was administered as the sodium salt and free acid (1:1) by constant intravenous infusion for 3 hours. At the start of the fourth hour sodium malonate† was administered intravenously as a single dose. The infusion of radioactive acetate was continued as before for another 3-hour period without interruption. By this procedure each animal acts as its own control for acetate oxidation. In the absence of excess carrier acetate the rate of acetate oxidation is constant, provided that the rate of acetate injection is constant. *Oxidation of malonate* (*Table II*). C^{14}-labeled sodium malonate was injected at

† Carboxyl labeled C^{14} disodium malonate was prepared from diethylmalonate purchased from Tracerlab, Inc. Methylene labelled C^{14} disodium malonate was prepared from dicalcium malonate purchased from Volk Radiochemical Co., Chicago, Ill.

TABLE II. Oxidation of C^{14} Labeled Malonic Acid after Single Intrav. Inj. Results expressed as mg/kilo/hr of malonic acid except in Exp. 476 where oxidation is expressed as % of inj. counts. Carboxyl labeled malonate was used in Exp. 476, 481, 478 and 480; and malonate-2-C^{14} was used in Exp. 509.

Hours	Exp. No. 476 Tracer	481 50	478 300	480 685	509 200
		Amt of malonic acid inj., mg/kilo			
1	5.0	3.0	7.9	18	2.7
2	8.5	3.4	13.4	17	3.3
3	6.5	2.8	13.5		2.9
4	5.9	1.9	8.1		3.5
5	3.7	1.5			3.7
Total % recovery	29.6	24	15		8
Total intracellular transfer of inj. dose (%)	58	55	60		

dosage levels (calculated as free acid) given in Table II. The expired CO_2 was collected each hour for C^{14} determinations. At the end of each experiment a plasma sample from each animal was also obtained for the C^{14} content. The total intracellular transfer of administered malonate was calculated from the counts administered and from the counts recovered in the terminal plasma samples. The extracellular space was assumed to be 22% of body weight. *Volume of distribution of injected malonate* (*Table III*). Plasma samples were taken at regular intervals after intravenous administration of radioactive malonate. These plasma samples were oxidized and the carbon collected and counted as $BaCO_3$.

Results. Effect of malonate administration on oxidation of acetate. It is apparent from the data in Table I that malonate ad-

TABLE III. Volume of Distribution of Injected Carboxyl Malonate. Results expressed as % of body wt (corrected for oxidation).

Time	Tracer Exp. 476	Amt of malonic acid inj., mg/kilo 50 Exp. 481	300 Exp. 478
20 min.	40	20	18
1 hr 20 min.	48	29	21
2 20		28	28
3 20	54	43	
4 20		33	37
5 20	65	34	

ministration in doses up to 250 mg/kilo does not inhibit oxidation of either tracer or carrier amounts of acetate.

Oxidation of malonate. The data in Table II show that both carboxyl and methylene C^{14}-labeled malonate are oxidized to CO_2 by the eviscerated animal. Rate of oxidation appears to be dependent on plasma concentration of malonate since the highest oxidation rate is found in those animals administered malonate at the higher dosage level. It should be pointed out that even in the animal with the highest malonate oxidation rate, the oxidation of malonate represents less than 2% of total metabolism of the animal. Intracellular transfer rate, which ranges from 55 to 60% over the 5-hour period of observation, suggests that cell entry is an important limiting factor in the rate of malonate oxidation. Dosages of malonate at 685 mg kilo and higher proved to be quite toxic to the animal. This toxicity did not appear to be due to an inhibition of the citric acid cycle, but rather to the possible binding of metal ions by malonate. One indication of this was the lack of blood coagulation in the eviscerated animals treated with malonate.

Volume of distribution of injected malonate. The data in Table III show that injected malonate circulates initially only in a volume equivalent to the extracellular compartment. The intracellular transfer rate of malonate increases slowly with time. We consider the extracellular compartment to be about 20-25% of body weight.

Discussion. It has been shown (Table I) that under our conditions malonate does not have any apparent effect on oxidation of acetate to CO_2 by the eviscerated-nephrectomized rabbit. The lack of effect of even higher doses of malonate on acetate oxidation had been noted previously in the intact rat(7). The inability of malonate to inhibit acetate oxidation can be explained by 2 findings presented in this paper. First, volume of distribution studies show that cell permeability barriers exist for malonate, for approximately 40-50% of the injected dose is still circulating in the extracellular compartment 5 hours after malonate administration. Next, oxidation studies demonstrate that both carboxyl and methylene carbons of malonate can be oxidized. Thus, it can be seen that a slow rate of malonate entry into the cells coupled with its oxidation by the tissues, prevents intracellular concentration of malonate from attaining a level high enough to inhibit a succinic dehydrogenase.

The ability of the eviscerated animal to oxidize malonate is not surprising. Lifson and coworkers(8,9) studying C^{13} carboxyl labeled malonate conversion in the intact mouse and rat demonstrated the degradation of malonate to carbon dioxide. More recently Hayaishi(10) has reported that rat kidney homogenates were capable of producing isotopic CO_2 from radioactive malonate. Homogenates from rat liver and muscle, however, were found to be inactive. From our results it is clear that tissues other than kidney are also able to oxidize malonate.

A finding of particular interest to us is that a concentration of malonate much less than that normally used to demonstrate malonate inhibition in intact animals, was found to be lethal to the eviscerated animals. If one assumes that administration of this compound results in a chelation of metal ions, then it would appear that the *in vivo* administration of malonate could result in non-specific metabolic alterations which would allow very little experimental value for elucidating metabolic pathways. The finding of succinate(5) and glucose accumulation(4) by others after malonate injection could very well be a demonstration of malonate inhibition in the classical sense; however, this accumulation may also be due to causes other than a strict inhibition of succinic dehydrogenase.

Summary. 1. Oxidation of acetate to CO_2 by the eviscerated-nephrectomized rabbit is unaffected by malonate. 2. Studies on the fate of carbon-14 labeled malonate indicate that the intracellular transfer rate of malonate is low. 3. Conversion of radioactive malonate to CO_2 has been demonstrated in the eviscerated-nephrectomized rabbit. 4. The slow rate of cell entry and subsequent oxidation of malonate is offered as a possible explanation for the non-inhibitory effect of malonate on acetate oxidation.

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Cultivation of Type 2 Dengue Virus in Rhesus Kidney Tissue Culture.* (22692)

SUSUMU HOTTA† AND CHARLES A. EVANS.
Department of Microbiology, University of Washington, Seattle, Wash.

It was reported(1) that mouse-adapted type 1 dengue virus of the Mochizuki and Hawaiian strains multiplied in trypsinized rhesus kidney tissue cultures, and produced a cytopathogenic effect. It was also demonstrated that degeneration of infected cells was inhibited by type specific anti-dengue immune rabbit serum, thus making possible virus neutralization tests in tissue culture. In the present paper, experiments are reported in which type 2 dengue virus was cultivated in tissue culture.

Materials and methods. Tissue cultures employed consisted of trypsinized rhesus kidney cells(2). Technical details including preparation and incubation of cultures, constituents of medium, etc., were described previously(1). Type 2 dengue virus of the New Guinea C strain was received on Jan. 25, 1955, from Dr. Edwin H. Lennette in the form of 20% mouse brain homogenate in 30% normal rabbit serum frozen with dry ice. It was stated that this material represented the 19th mouse brain passage, and that its LD_{50} titer was $10^{-6.2}$ at time of harvest, Nov. 11, 1953. The virus was kept in dry ice chest until used. On Feb. 1, 1955, the frozen material was thawed at room temperature, and diluted with Hanks' balanced salt solution. Its infectivity was measured by injecting 2 to 3-week-old white mice intracerebrally with 0.02 ml of 10-fold dilutions. The mortality ratios of injected mice were 4/4 for the 10^{-3} dilution and 0/4 each for dilutions of 10^{-4} through 10^{-8}.

Results. Serial passage through tissue culture: Seven cultures were inoculated with 0.2 ml of above-mentioned virus diluted 10^{-3}. After 10 minutes 1.8 ml of culture medium was added to each tube. The cultures were incubated in the stationary state at 35°C. Half or whole volume changes of medium were made usually every 2 to 3 days, to keep the pH of the fluid phase in the range of 7.2 to 7.6. Eleven days after beginning of incubation a portion of culture fluid was taken from each tube and pooled with fluids from other similar tubes. 0.2 ml of the mixture was then inoculated into new cultures with subsequent addition of 1.8 ml of medium to each tube. In this manner serial passages through tissue cultures were performed at intervals of 7 to 15 days. The virus content of the inoculum for each subculture was assayed by injecting 0.02 ml of 10-fold dilutions into mice. The results obtained are summarized in Table I. The specimens used for tissue culture passage and virus titration contained no agent cultivable on ordinary bacteriological culture media.

The virus was cultivated in this tissue culture system for 18 passages during 176 days.

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† Present address: Microbiological Institute, Kyoto University, Kyoto, Japan.