the initial response of animals immunized with BGG-bentonite. The unidentified components, U_1 and U_2, were only detected in serum of hyperimmunized animals. Immunization with BSA or BGG in saline produced only 2 sera from hyperimmunized animals which contained detectable antibody, and in both cases only γ_1 antibody was present. Recently, Dixon *et al*(7) reported that immunization of rats with keyhole limpet hemocyanin in saline resulted in a 19 S antibody response; whereas, administration of this antigen in incomplete Freund's adjuvant produced a predominantly 7 S antibody response. Thus it seems that the sequence of the appearance of these antibodies of different immunoglobulin class in the rat is in part dependent on the nature of the antigen and the method of immunization.

Summary. By radioimmunoelectrophoresis, antibodies associated with γ_1, γ_2 and γM globulins and 2 unidentified components, U_1 and U_2, were detected in rat antisera to BSA and BGG. When rats were immunized with these antigens adsorbed to bentonite or included in complete Freund's adjuvant, the sequence of the immune response in rats involves first the formation of γ_1 antibody followed upon further immunization by γ_2 antibody. Generally, γM and the unknown components, U_1 and U_2, only appeared in hyperimmune sera.

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Base Composition and Thermal Denaturation of DNA Isolated from *Anaplasma marginale*.* (32019)

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Although DNA and RNA occur in *Anaplasma marginale,* as confirmed by histochemical staining of infected erythrocytes (RBC)(1,2,3), quantitative data are not available on base composition and thermal denaturation. The present investigation was undertaken to isolate the nucleic acids and determine their base composition However, only DNA was found and a study of its thermal denaturation properties was also conducted.

Materials and methods. Experimental calves. Splenectomized calves were maintained and infected with *A. marginale* as previously described(4).

Collection of infected blood. When the infected RBC count reached 50 to 80%, as determined by Giemsa staining of blood smears, the calves were exsanguinated and the blood collected in heparin sodium solution (1,000 U.S.P. units/ml—0.3 ml/50 ml blood). The RBC were separated from plasma and buffy coat and washed 4 to 5 times with 0.9% NaCl solution by centrifuging at 1,060 $\times$ *g* for 20 min at 4°C.

Isolation of intact, cell-free Anaplasma *bodies.* Equal volumes of 0.9% NaCl solution containing 0.1 M sodium citrate were added to packed RBC and the suspensions, in 50-ml volumes, sonicated for 70 seconds

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TABLE I. Mean Percent Base Composition of DNA Isolated from *Anaplasma marginale* by 2 Methods.

Method of isolation and reference	No. of different isolates	Relative percent base*			
		Adenine	Guanine	Cytosine	Thymine
Hotchkiss(6)	8	31.76 ± .93	33.02 ± 1.45	17.72 ± .70	17.48 ± 1.77
Marmur(7)	6	32.70 ± 1.38	34.35 ± .92	15.50 ± 2.20	17.80 ± 1.85

* Mean percent base ± S.D.

employing a Branson Sonifier at 8 amps. The sonicates were centrifuged at 1,060 × *g* for 20 minutes at 4° to remove large particulate matter and the recovered supernatant fluids centrifuged at 27,000 × *g* for 20 minutes at 4°C. The resulting sediments were washed 4 times in phosphate-buffered 0.9% NaCl solution at pH 7.0 by centrifuging at 27,000 × *g* for 20 minutes at 4°C and the final pellets examined for contaminating RBC, leucocytes, and reticulocytes. DNAase and RNAase (0.05 mg/10 ml suspension) were added to the pellets suspended in 10 ml of 0.9% NaCl solution and incubated for 15 minutes at 37°C to remove nucleic acids which may have been liberated by the destruction of contaminating leucocytes or reticulocytes during sonication of washed RBC. The suspensions were finally washed by centrifugation as above to remove residual enzymes and the *Anaplasma* bodies were stored fresh at 4°C or frozen at —22°C after suspending in 10-15 ml of 0.9% NaCl solution containing 0.1 M sodium citrate.

Sucrose density gradient centrifugation. To insure that these preparations were further devoid of contaminating cellular debris, suspensions of *Anaplasma* bodies were subjected to sucrose density gradient centrifugation as described by Rogers *et al*(5).

Isolation, hydrolysis, and quantitation of DNA. The methods of Hotchkiss(6) and Marmur(7) were each separately employed to isolate DNA from the purified organisms. Hotchkiss' method was also used for isolation of DNA from organisms which were purified by sucrose density gradient centrifugation.

DNA in 15.6-mg amounts was hydrolyzed for 1 hour according to the technique described by Wyatt(8). The hydrolysates were chromatographed and quantitated employing the method of Benedich(9). Base standards of adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U) were also chromatographed and blank lanes served as controls.

Attempts to isolate nucleic acids from normal bovine RBC were also made. Furthermore, the method of Hotchkiss was employed in attempts to isolate RNA from suspensions of *Anaplasma* bodies.

Thermal denaturation of DNA. Anaplasma DNA, which had previously been freeze-dried, was dissolved in 1 M NaCl-0.1 M sodium citrate solution, and the preparation equally divided into 5 tubes. Each sample was maintained at the temperatures indicated in Table II for 10 minutes, cooled to —70°C, and then allowed to return to ambient temperature (23°C). Readings were made in a spectrophotometer at 260 mμ using 1 M NaCl-0.1 M sodium citrate solution as reference. Highly-polymerized DNA (Nutritional Biochemicals Co.) represented standard double-stranded DNA.

Results. DNA from suspensions of *A. marginale* was isolated by 2 methods(6,7). Analyses of bases in these samples indicated that both techniques yielded DNA with approximately similar compositions (Table I). Molar ratios of A/T and G/C for DNA isolated by the method of Hotchkiss(6) were 1.9 and 2.5, respectively, whereas A/T and G/C were 1.9 and 2.8, respectively for DNA isolated by the procedure described by Marmur(7).

All attempts to isolate RNA(6) and to detect U in the hydrolysates were unsuccessful. Furthermore, nucleic acids could not be isolated from suspensions of washed normal bovine RBC.

Base analysis of DNA isolated by the method described by Hotchkiss(6) from frozen suspensions of the organism purified further by sucrose density gradient centrifu-

TABLE II. Optical Densities at 260 mu of Heated DNA from *Anaplasma marginale* and Highly-Polymerized DNA.

Source of DNA	Temperature (°C)	Optical density
A. marginale	22.4	.97
	47.0	.97
	60.0	1.00
	80.0	1.00
	100.0	1.00
Highly-polymerized	20.0	.25
	40.0	.39
	60.0	.55
	80.0	.71
	100.0	.71

gation gave average values of 32.1%, 33.9%, 16.5%, and 17.5% for A, G, C, and T, respectively. In fresh suspensions the average percent composition for A, G, C, and T was 31.6, 34.5, 16.7, and 17.2, respectively.

A significant increase in hyperchromicity was not displayed in thermal denaturation studies by DNA isolated from *A. marginale* as was shown with highly-polymerized DNA (Table II).

Discussion. The results of base composition analyses suggest that DNA from *A. marginale* is single-stranded. The molar ratios for A/T and G/C are 1.9 and 2.5, respectively, for DNA isolated by the method of Hotchkiss(6). The isolates obtained using Marmur's(7) procedure have ratios of 1.9 and 2.8 for A/T and G/C, respectively. The suggested single-stranded nature of *Anaplasma* DNA is again exemplified by the results of the thermal denaturation experiments where a significant rise in optical density did not occur with an increase in temperature.

The suggestion that *Anaplasma* DNA is single-stranded finds prominence on 2 points. A life cycle has not been found from which one may assume that it is single-stranded when present in the erythrocyte. Furthermore, being highly parasitic, *Anaplasma* may not need a large double-stranded DNA molecule. However, there may be a double-stranded form in its pre-erythrocytic stages. Preliminary data from this laboratory indicate the presence of the organism in reticuloendothelial cells prior to its appearance in RBC.

The sonication process which is employed to release the organism from RBC may be subject to criticism as possibly disrupting the DNA, although lysis of the agent does not occur as observed by light and electron microscopy. Furthermore, if DNA was double-stranded subsequent isolation and quantitation would yield molar ratios of approximately 1 for A/T and G/C.

The absence of RNA in *A. marginale* was unexpected because of earlier reports confirming its presence by histochemical staining of infected RBC(1,2,3). However, the present report deals with purified preparations of the organism which were also treated with DNAase and RNAase prior to extraction. This treatment would have removed possible contaminating nucleic acids from such cells as leucocytes and reticulocytes which may have been carried over during purification of *A. marginale*. It is not known if the nucleic acids of the organism were affected by the action of these enzymes. Rogers and Dimopoullos(10) have shown that the agent contains a lipoprotein coat which is responsible for serologic activity. DNAase and RNAase did not affect this activity and therefore, the lipoprotein may protect the nucleic acid of the organism from this action.

Summary. DNA, isolated by 2 methods from purified *A. marginale,* was found to possess average molar ratios of A/T and G/C of 1.9 and 2.65, respectively. There was also a lack of increase in hyperchromicity of isolated DNA in thermal denaturation studies. Attempts to isolate RNA or to detect U were unsuccessful.

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In vivo Fatty Acid Synthesis in Adipose Tissue and Liver of Meal-Fed Rats.* (32020)

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Tepperman *et al*(1) observed increased RQ values in rats restricted to a single daily meal period (meal-fed), suggesting enhanced lipid synthesis. Subsequent studies by these workers and others demonstrated significantly greater incorporation of ^{14}C-labelled substrates into fatty acids by liver slices from meal-fed as compared to slices from control rats(2). More recently meal-feeding has been shown to induce even more dramatic increases in fatty acid synthesis in rat epididymal adipose tissue(3-5).

In vitro data on the conversion of radioactive substrates to fatty acids and the original RQ measurements reported by Tepperman *et al*(1) imply that fatty acid synthesis is enhanced in the intact meal-fed rat but this has not been demonstrated. Consequently an experiment was designed to study fatty acid synthesis *in vivo* and also to obtain information on the relative importance of adipose tissue and liver as sites of fatty acid synthesis. The results of this investigation form the basis for the present report.

Methods. Male Holtzman rats weighing about 220 g were used. The animals were housed singly in metal cages having raised wire floors and in a temperature controlled room. A commercial rat diet† in powdered form was fed throughout this study.

The rats were divided into 2 equal groups on the basis of body weight. One group was fed *ad libitum* (nibblers) and the others had access to food from 8 A.M. to 10 A.M. only (meal-eaters). The animals were maintained on these regimens for 2 weeks, a period shown to be adequate for induction of enhanced lipogenesis and the accompanying enzymatic changes(6). Water was available at all times and food consumption and body weights were determined at weekly intervals. The nibbling animals were fasted from 10 A.M. on the day preceding the experiment until 8 A.M. on the day of the experiment as were the meal-fed animals. At this time both meal-fed and nibbling animals were given 10 g of food, an amount which all animals rapidly consumed. At the start of the feeding period each animal received 4.5 μc of glucose-U-^{14}C in 0.1 ml of physiological saline intraperitoneally. The radioactive glucose had a specific activity of 3.46 μc/μmole and consequently an insignificant amount of glucose was given (234 μg per rat). Four rats from each group were killed 3, 6 and 9 hours after the start of the meal period. The animals were decapitated, blood was collected and the necessary tissues were rapidly removed and weighed. The epididymal adipose tissue was placed in chloroform:methanol (2:1, v:v) to extract the lipids, liver lipids were extracted with the same solvent. The lipid extracts were washed by the method of Folch *et al* (7) to remove non-lipid radioactive materials and the solvent was evaporated. The residual lipid was saponified by refluxing with 10 ml of 5% ethanolic KOH, an equal volume of water was then added and the non-saponifiable lipids were removed by three successive extractions with petroleum ether (BP-30-60°). The aqueous phase was acidified with HCl and the fatty acids were removed by repeated extraction with petroleum ether. The ether extracts were combined in liquid scintillation vials, the solvent was evaporated

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† Rockland mouse/rat diet (complete), Teklad, Inc., Monmouth, Ill.