utes, all heavy RNA is partially degraded and can be found as a large heterogenous peak in the lighter part of the gradient, between 4 and 10 s. The yields of RNA extractable from the nuclei at pH 7.6 or pH 8.3 decrease from 0.7 mg/10 g of liver to 0.4 mg/10 g after an incubation of only 15 minutes. Dukes and Sekeris used incubation periods of 2 or even 3 hours. The cortisol concentrations which stimulated the incorporation of uracil into isolated nuclei in their experiments were about 500 times as high as the normal non protein-bound plasma concentration. Compared to the effects obtainable by application of the hormone *in vivo,* the stimulation of incorporation reported by Dukes and Sekeris is still small. For these reasons it seems doubtful that their results reflect a physiologically relevant mechanism. The degree of normal structure which must be maintained by a subcellular system in order to respond to a hormonal stimulus in a specific way remains unsettled.

Summary. Nuclei isolated from rat liver cells can incorporate H^3-cytidine triphosphate into RNA *in vitro.* Cortisol added to the incubation medium is taken up by the nuclei in small amounts, but does not stimulate nuclear RNA synthesis. Administration of the hormone *in vivo,* however, results in a marked increase in incorporation of H^3-cytidine triphosphate incubated *in vitro* with nuclei isolated from 20 to 180 minutes after the injection. Synthesis of RNA *in vitro* is accompanied by a simultaneous partial degradation of RNA which affects the yields of RNA extractable from nuclei even after very short periods of incubation.

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The Nature of *Macacus rhesus* Erythrocyte Agglutinins Found in the Sera of Hepatitis Patients. (31268)

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Hoyt and Morrison(1) reported that sera of patients with infectious hepatitis agglutinated *Macacus rhesus* erythrocytes. It was suggested by Rubin *et al*(2) that the hemagglutination resulted from a specific effect of hepatitis virus, whereas Hoyt *et al*(3) suggested that the hemagglutination mechanism involved a gamma globulin of the sera of hepatitis patients. This report concerns our studies on the agglutinating component found in the sera of hepatitis patients.

Methods. Procedures for the agglutination test were essentially similar as described by Hoyt and Morrison(1). *Macacus rhesus* erythrocytes were washed 3 times with 0.9% saline and a 2% suspension prepared. Serial 2-fold dilutions of the sera, previously inactivated at 56°C for 30 minutes, were made in 0.2 ml volumes and 0.2 ml of the erythrocyte suspension was added to each. After incuba-

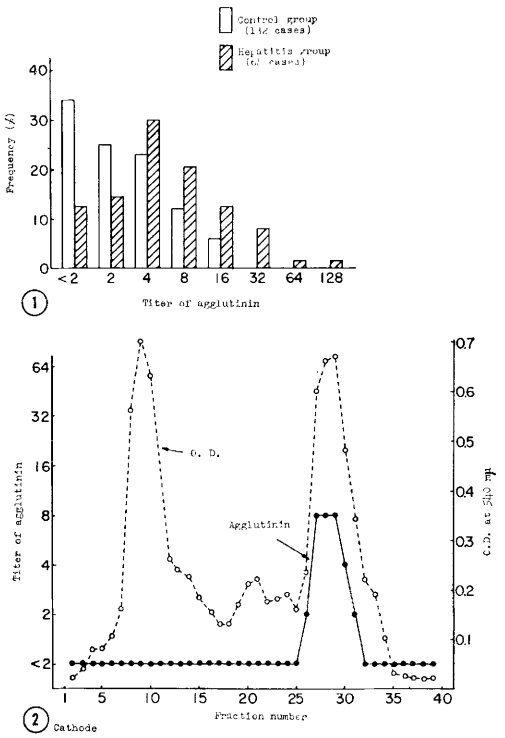

FIG. 1. Agglutinin titers in sera of hepatitis patients and healthy persons.

FIG. 2. Fractionation of agglutinins in the serum of hepatitis patient by starch gel electrophoresis. The electrophoresis was conducted on starch block ($7 \times 1.5 \times 40$ cm) with $\frac{M}{30}$ phosphate buffer of pH 8.3 and 3.8 mA/cm^2 for 17 hr. Each fraction was eluted with 4 ml of 0.01 M phosphate buffer plus 0.14 M NaCl of pH 7.2. A portion of the fractions was taken for Biuret reaction and O.D. at 540 mμ was read. The remainder was used for agglutinin titration.

tion at 37°C for 60 minutes, tubes were centrifuged at 1,000 rpm for 2 minutes and the agglutination pattern was read after brisk shaking. The titer of agglutinin was expressed as the reciprocal of the highest serum dilution which yielded macroscopic hemagglutination.

Results and discussion. The sera of 64 infectious hepatitis patients, whose diagnoses were characterized by laboratory examinations, histological findings of biopsy materials and clinical symptoms, were obtained. For control, sera obtained from healthy persons were used. Fig. 1 illustrates the distribution of the agglutinin titers among patients and controls. The median titer among hepatitis patients (1:4) is significantly higher than that of the control group (<1:2). Approximately 88% of the patients had measurable titers as opposed to 65% of the controls. On the basis of these observations attempts were made to define the nature of the agglutinins produced in hepatitis patients.

An analysis of the sedimentation properties of the hemagglutinins was carried out by sucrose gradient (15-40%) centrifugation with the Hitachi 40 P ultracentrifuge. Similar results with 3 sera of hepatitis patients indicated that the agglutinins had an S value of less than 40. By means of starch zone electrophoresis, as shown in Fig. 2, the agglutinin was found in fractions corresponding to gamma globulin. This was observed with sera of 2 hepatitis patients. Hemagglutination titers were the same when the erythrocyte and serum mixture was incubated at 4°C, 20°C, and 37°C. The agglutinin could be removed by adding packed erythrocytes, a procedure which concomitantly resulted in removal of gamma globulins. The agglutinins adsorbed to the erythrocytes cannot be eluted by varying temperature. This body of preliminary evidence suggests that the hemagglutinin is antibody rather than viral.

The nature of agglutinin was further examined by testing the heat stability and sensitivity to 2-mercaptoethanol (2-ME)(4). Tests were performed on gamma globulin fractions of a patient's serum obtained by zone electrophoresis shown in Fig. 2. As shown in Table I, the agglutinins disappeared after heating at 70°C for 30 minutes, but not at 56°C. After the treatment with 2-ME, the agglutinins were no longer detectable. It was confirmed that agglutinins in sera of 3 other

TABLE I. Effect of 2-Mercaptoethanol (2-ME) and Heat on Hemagglutinin. Heating was at the indicated temperatures for 30 min. Treatment with 0.1 M 2-ME was carried out at 37°C for 2 hr and dialyzed against 0.14 M NaCl.

Fraction No. in the zone electrophoresis (Fig. 1)	Agglutinin titer			
	Non-treated	Treated with 2-ME	Treated with 56°C	Treated with 70°C
27	8	<2	8	<2
28	8	<2	8	<2
29	8	<2	8	<2

hepatitis patients were also inactivated by these treatments.

From these studies, the nature of hemagglutinins against *Macacus rhesus* erythrocytes found in the serum of hepatitis patients appears to be 19 S antibody. The macroglobulin nature of this agglutinin was further confirmed when it was detected in the first cut of serum passed through the column of Sephadex G-200(5).

Detailed studies are under way exploring the hypothesis that the *Macacus rhesus* hemagglutinins are actually autoimmune antibodies.

Summary. Agglutinin titers for *Macacus rhesus* erythrocytes were higher in sera of infectious hepatitis patients than those of normal persons. The agglutinin was found to be a macroglobulin antibody (19 S), as shown by ultracentrifugation, zone electrophoresis, gel-filtration and sensitivity to heating and mercaptoethanol.

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Effect of Condensed Phosphates on Vitamin D-Induced Aortic Calcification in Rats.* (31269)

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It has been shown(1) that inorganic pyrophosphate and longer chain condensed phosphates inhibit calcium phosphate precipitation *in vitro* at concentrations as low as 10^{-6} M. Subsequently pyrophosphate was found to be a normal constituent of urine(2) and plasma(3), and it was suggested that this substance might be of importance in preventing collagen and other nucleating substances from calcifying(3,4). In favor of this hypothesis was the finding that pyrophosphate inhibited mineralization of chick embryo femurs grown in tissue culture(5). Further work(6,7) showed that pyrophosphate or polyphosphates inhibit calcification of the aorta induced by massive doses of vitamin D *in vivo*. In the present study we have attempted to investigate the mechanism of this inhibition of aortic calcification in more detail.

Material and methods. The experimental design was based on that of Gillman *et al*(8) who obtained heavy calcification in the aortas of rats after dosage with a large amount of vitamin D_2. Forty-eight female Wistar rats, weighing from 200 to 250 g and fed stock rat chow (Altromin, Lage, Germany) throughout, were divided into two groups. One group received daily subcutaneous injections of Graham salt, a long chain polyphosphate (J. A. Benckiser, Ludwigshafen/Rhein, Germany) in saline at pH 7.4, given at a dose level of 10 mg P/kg body wt, over the 15 days of the experiment. The other group did not receive Graham salt. From the third to the seventh days inclusive, both groups were

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