molabile agent, such as *R. prowazekii*, centrifugation plays the dual role of accelerating adsorption and, by this action, of preserving activity of a greater number of particles.

During development of the centrifugation procedure, a number of arbitrary choices were made which require further testing. For example, speed of centrifugation was the highest which could be conveniently employed, but the effect of higher or lower speeds has not been investigated. Temperature during centrifugation is another variable which was eliminated by selecting 20°C. This choice was made as a compromise to prevent both thermal inactivation of infectious agents and inhibition of host-parasite interaction.

The practical advantages of the procedure are obvious. It is simple and efficient and produces consistent results that closely resemble those obtained in eggs. It also offers an opportunity to separate adsorption from the other phases of intracellular growth of the infectious agents. Similarity of results obtained with 4 different agents and 2 unrelated host cells indicates that this method may have wide applications.

Summary. A method for effective inoculation of cultured cells with some rickettsiae and larger viruses is described. Chick embryo entodermal cell monolayers were prepared either in standard screw-cap tubes or, when microscopic examination was required, in flat-bottomed tubes containing 12 mm round coverslips. Following addition of inoculum diluted in fresh nutrient, the tubes were centrifuged horizontally at 1600-1800 g for 1 hour at 20°C. By this procedure infection of cells with *Rickettsia prowazekii* was greatly enhanced (about 10,000-fold) over that obtained in stationary tubes, and susceptibility of entodermal cells appeared to be as great in centrifuged tubes as in intact embryo. Good enhancement was also obtained with psittacosis (about 100-fold) and trachoma (at least 10-fold) viruses and moderate enhancement with *Coxiella burnetii*. The same procedure was successfully used to enhance infection of cells from human skin cell line (HuS 2806) with psittacosis virus.

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Effect of Cortisone on Liver Phosphorylase Activity.* (25638)

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Liver glucose-6-phosphatase activity, in adrenalectomized rats, is increased by cortisone(1,2). While this could explain increase in blood sugar induced by cortisone, it would not explain increased liver glycogen also induced. Phosphorylase is required for synthesis of glycogen in the glycolytic pathway. The effect of adrenalectomy and subsequent cortisone injection on rate of phosphorylase activity in rat liver has been determined.

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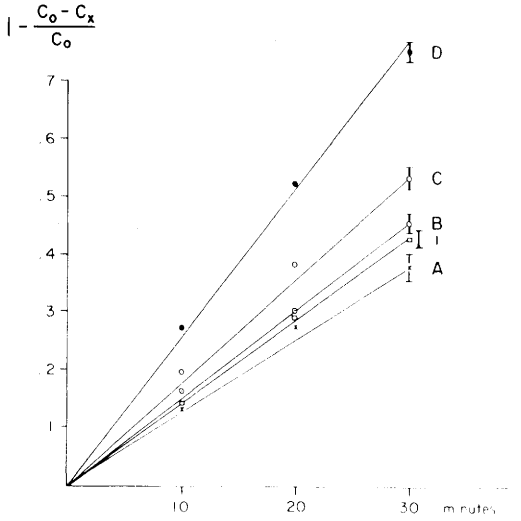

FIG. 1. Liver phosphorylase activity. C_o, mg P in substrate/mg N; C_x, mg P liberated/mg N; A, adrenalectomized, 1 intact control; B, 1.25 mg cortisone; C, 2.5 mg cortisone; D, 5 mg cortisone. Upper and lower limits of 30 min. values are given.

Methods. Intact and adrenalectomized female rats 125-150 g, fasted for 24 hours, of both Wistar and Sprague-Dawley strains, were used, 4-6 animals of each strain in each experiment. No strain differences were noted. Cortisone acetate, 1.25, 2.5 or 5 mg/100 g, was injected intramuscularly at the end of 24 hour fast and animals were sacrificed 24 hours later. Controls were sacrificed after a 48 hour fast. Livers were removed, analyzed immediately for phosphorylase activity by the method of Nimni *et al.*(3) and for glycogen by the method of Good *et al.*(4). Phosphorylase was determined after 10, 20, and 30 minutes for incubation. Activity was expressed as mg P liberated per mg N. Rate was determined by $1-\frac{C_o-C_x}{C_o}$ plotted against time, when C_o is mg P in substrate/mg N and C_x is mg P liberated/mg N.

Results. Fig. 1 shows liver phosphorylase rate changes induced by adrenalectomy alone and subsequent cortisone acetate injection. Average liver glycogen content of intact fasted controls was 0.14% and of adrenalectomized controls 0.10%. Average glycogens of adrenalectomized rats given 5.0, 2.5 and 1.25 mg cortisone acetate were 1.60, 0.68 and 0.31% respectively. Adrenalectomy decreased rate of liver phosphorylase activity below that of fasting intact animals. Cortisone increased rate of activity above that of controls; 1.25 mg gave a rate slightly, but not significantly greater, than that of intact fasted animals. Liver glycogen on the other hand, at this cortisone level, was significantly elevated over the fasting control.

Sorensen(5) reported a decrease in liver phosphorylase in fasted as compared with fed rats, both intact and adrenalectomized. Increases in liver phosphorylase activity were reported in obese hyperglycemic mice(6) and in rats during glucose absorption(3).

Since cortisone increases activity of both phosphorylase and glucose-6-phosphatase it probably also increased the activity of other enzymes that feed carbon chains into the glycolytic cycle. This would be necessary to produce an increase in both glucose and glycogen. Rosen *et al.*(7) and Gravasto *et al.*(8) found that hydrocortisone increases activity of glutamic-pyruvic and glutamic-oxalacetic transaminases respectively. These enzymes could provide, from protein, the necessary carbon chains for the glycolytic cycle since it is well known that peripheral protein is decreased by cortisone(9,10). Mark and Horecker(11) presented evidence that alternate pathways are present for glycogen synthesis but found that the glycolytic mechanism was the major one. One such pathway was recently discovered by Leloir and Cardini(12) who found a liver enzyme system that synthesizes glycogen from uridine-diphosphoglucose. The increases in phosphorylase and glucose-6-phosphatase activity may actually be secondary to transaminase activity and represent a response to increase in glucose precursors.

Summary. Rate of liver phosphorylase activity is decreased in fasted, adrenalectomized rats. Cortisone acetate administered at levels of 5, 2.5 or 1.25 mg/100 g body weight increased this activity above that of controls, the latter dosage only slightly above control level. Since glucose-6-phosphatase activity is also reported to be increased it is suggested that these 2 enzymatic effects may represent response to increased production of glucose precursors from peripheral protein.

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Zone Electrophoresis Studies on Hemagglutinin of Hemagglutinating and of Masked Strain of Polyoma Virus. (25639)

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Eddy *et al.*(1) demonstrated an hemagglutinin in mouse embryo tissue culture fluids infected with polyoma virus. This hemagglutinin was lacking in cultures with no tumor-producing capacity. It was not possible to dissociate hemagglutination from the cytopathogenicity and tumor-inducing capacity by various methods such as heat treatment, ether treatment, limiting dilution titration, and first passage plaques from limiting dilutions. The authors, therefore, considered hemagglutination to be an inherent characteristic of the tumor-inducing agent. Kahler *et al.*(2) found in electron microscopic studies of purified polyoma virus an apparently uniform population of particles with a diameter of 40-45 mμ. By sedimentation experiments in a zonal density gradient particle counts were found to parallel the hemagglutination titer. However, no data on the relation of number of particles observed to possible tumor-inducing capacity are given. Hartley and Rowe (3) showed that culture fluids with high tumor-inducing capacity might fail to produce hemagglutination. It could be demonstrated, however, that unmasking of the hemagglutinin could be achieved either by heat or RDE treatment. It seemed desirable to study the interrelation of the different biological attributes of mouse polyoma issue culture fluids by methods other than sedimentation. Preliminary results of electrophoretic studies on the hemagglutinin both of hemagglutinating and of non-hemagglutinating (masked) Polyoma strains are given. Comparable studies on electrophoretic mobility of tumor-inducing capacity, and of cytopathogenicity are in progress.

Materials and methods. Virus. Strain 1000 with a hemagglutination titer of 1/128 and strain 8844 with a titer ¼ both from the Nat. Cancer Inst. were used. Both strains were known to give about 100% early tumor formation in Swiss mice. Virus was grown in 2% horse serum in medium 199. For unmasking the hemagglutinin of strain 8844, heat treatment for 30 to 60 min. in 56°C water bath was used. *Electrophoresis technic.* A detailed description of the electrophoresis apparatus and technic has been given(4,5,6). Tissue culture fluids were treated with one cycle of centrifugation for 10 min at 6000 g in a Spinco rotor 40. The supernate was dialysed against the buffer used for electrophoresis and mixed with the appropriate quantity of cane sugar to fit the density conditions for introduction into the electrophoresis column. Veronal buffer of pH 8.6 containing 205 g sodium diethylbarbiturate and 28 g diethylbarbituric acid in 10 l of distilled water was