interferon synthesis occurred when higher multiplicities of virus were employed.

Discussion. The amount of interferon produced by chick embryo cell cultures infected with Semliki Forest virus was dependent upon the multiplicity of infection as previously shown for chikungunya virus in the same type of cell system(1). Optimal yield of interferon was obtained with a different cell-virus ratio than previously found for chikungunya virus(1). It is possible that the "ideal" multiplicity of infection might differ from 1 preparation to another of the same virus. Studies which compare the yield of interferon by different viruses should take this possibility into consideration. The inhibition of interferon synthesis at high multiplicities of virus was more marked with Semliki Forest virus than for chikungunya virus. A similar phenomenon has been shown for rat tumor cells infected with Sindbis virus(6) and dog-kidney cells infected with herpes simplex virus(7). Contrariwise, Burke and Isaacs(8), using ultraviolet-inactivated influenza virus and chick chorioallantoic membranes, did not find any inhibition of interferon production when high doses of non-replicating virus were employed. Furthermore, chikungunya virus preparations usually induced less interferon in chick cell cultures than did the same dilution of the virus preparation in which the plaque forming ability had been lost by pre-incubation at 35° for 23 hours(9). These findings suggested that active plaque forming virus inhibited the production of interferon and may be related to the "inverse interference" phenomenon described by Lindenmann (10). It is likely that those viruses which rapidly shut off host RNA and protein synthesis would not be able to induce interferon synthesis in such a system as suggested by Aurelian and Roizman(7), Taylor(11), and by Wagner and Huang(12). Quantitative differences in the ability of viruses to induce interferon synthesis may be related to the rapidity and extent of this inhibition of cellular macromolecular synthesis and also to whether the "inactive" virus can also accomplish this cellular inhibition.

Summary. The yield of interferon in chick embryo cell cultures infected with Semliki Forest virus is dependent upon the multiplicity of virus employed. Optimal yields of interferon were found when a multiplicity of about 0.2 was used. Higher multiplicities of virus resulted in decreasing yields of interferon.

1. Gifford, G. E., Nature, 1963, v200, 91.
2. Gifford, G. E., Mussett, M. V., Heller, E., J. Gen. Microbiol., 1964, v34, 475.
3. Lindenmann, J., Gifford, G. E., Virol., 1963, v19, 283.
4. ———, ibid., 1963, v19, 302.
5. Riley, B. P., Toy, S. T., Gifford, G. E., Proc. Soc. Exp. Biol. & Med., 1966, v122, 1142.
6. DeMaeyer, E., DeSomer, P., Nature, 1962, v194, 1252.
7. Aurelian, L., Roizman, B., J. Mol. Biol., 1965, v11, 539.
8. Burke, D. C., Isaacs, A., Brit. J. Exp. Path., 1958, v39, 78.
9. Gifford, G. E., Heller, E., Nature, 1963, v200, 50.
10. Lindenmann, J., Z. Hyg. Infektionskrankh., 1960, v146, 287.
11. Taylor, J., Virol., 1965, v25, 340.
12. Wagner, R. R., Huang, A. S., ibid., 1966, v28, 1.

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Suppression of Plasma Immunoreactive Insulin by Epinephrine in Obese Subjects.* (32571)

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Hyperinsulinism is one of the metabolic characteristics of obesity as evidenced by increased plasma insulin concentrations in obese

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subjects in the fasting state(1) and after glucose challenge(1,2,3). According to current concepts, stimulation of alpha adrenergic receptors inhibits insulin secretion while beta receptor stimulation enhances insulin secretion(4). Bogdonoff and associates(5) have postulated that adrenergic receptors (beta) are relatively inactive in obesity as indicated by slow return to normal of the diastolic blood pressure after exercise and decreased free fatty acid mobilization. Sluggish alpha receptor mechanisms could theoretically cause the hyperinsulinism of obesity. This investigation examines the possible role of the adrenergic nervous system in the hyperinsulinism of obesity. The suppression of plasma immunoreactive insulin (IRI) during adrenergic receptor stimulation with epinephrine was compared in obese and nonobese subjects.

Methods and materials. Thirteen volunteer male medical students were selected for this study. Six were classified as nonobese and 7 as obese. Each obese subject was at least 20% over the theoretical ideal weight(6), the maximum ideal weight for large-framed individuals being used as a reference. Skinfold thickness in the mid-axillary line at the level of the xiphoid was determined with a Lange skinfold caliper in each subject. The range of skinfold thicknesses was 9 to 15 mm in nonobese subjects and 33 to 41 mm in obese subjects. None of the subjects had a family history of diabetes.

Following an overnight fast, each subject reported to the experimental room between 7 and 8 a.m. After the subject had been reclining for 15 minutes the first blood sample (F_1) was obtained. Immediately afterwards, an epinephrine infusion at a rate of 5 μg per minute was delivered by a Sage constant infusion pump through a 20 gauge needle in the antecubital vein. Five minutes later, a second blood sample (F_2) was obtained from an indwelling needle in the opposite antecubital vein. Immediately after the second blood sample had been taken, 25 g glucose (50 ml of 50% glucose in water) was injected intravenously over a 4 minute period. Blood samples were obtained every 5 minutes during the next 20 minutes while epinephrine was being infused and for 15 minutes after the infusion had been discontinued.

The 6 nonobese subjects were retested in an identical fashion several weeks later after 4 days preparation with 10 mg prednisone 3 times daily.

All blood samples were collected in heparinized tubes. Protein-free filtrates for blood glucose were made from 0.2 ml blood immediately after the blood samples had been obtained. Blood glucose was determined by a glucose oxidase method(7). Plasma insulin was determined by a modification(8) of the Yalow and Berson radioimmunoassay procedure using Insulin-^{125}I and separating free from bound insulin on Sephadex G-75.

Results and discussion. Plasma insulin concentrations in 7 obese and 6 non-obese subjects during and after epinephrine infusion are shown in Fig. 1. To assure equally potent

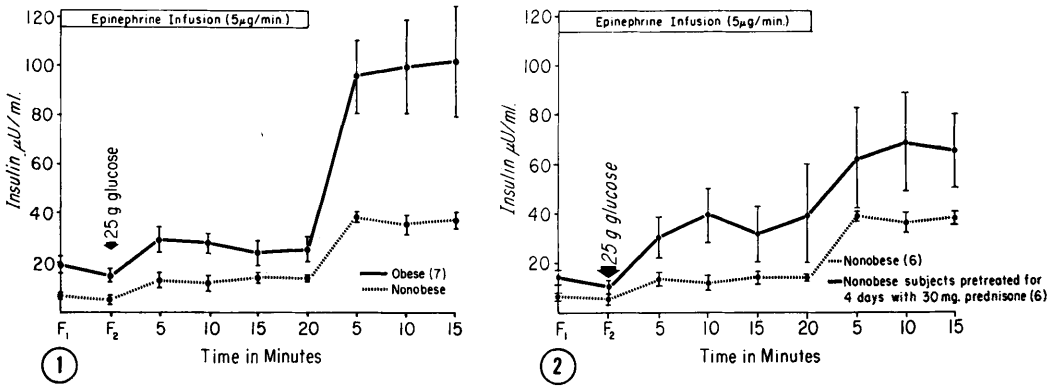

FIG. 1. Influence of epinephrine infusion on plasma insulin levels after intravenous glucose in obese and non-obese subjects. Means ± S.E.M. are shown.

FIG. 2. Influence of epinephrine infusion on plasma insulin levels after intravenous glucose in non-obese subjects with and without perdnisone pretreatment. Means ± S.E.M. are shown.

hyperglycemic stimuli in all patients, 25 g glucose was given intravenously 5 minutes after the start of the epinephrine infusion. Plasma insulin concentrations were higher in obese than in non-obese subjects throughout the experiment. The difference was significant with P values less than .01 or .02 at each time interval except at 15 and 20 minutes during the infusion.

The degree of suppression of plasma IRI by epinephrine can best be ascertained by comparing IRI values during and after the epinephrine infusion. Without epinephrine suppression, peak insulin concentrations normally occur within 2 minutes after intravenous glucose administration. The elevation in plasma IRI after the epinephrine infusion had been discontinued is presumably due to persistent hyperglycemia and would reflect the rise which would have occurred immediately after glucose administration had no epinephrine been given. In non-obese subjects, mean plasma IRI after cessation of the epinephrine infusion was 291% of the mean IRI concentration during the infusion; in obese subjects, the mean post-infusion IRI concentration was 374% of the mean value during the infusion. Although plasma IRI values in obese subjects never reached the low concentrations observed in non-obese subjects during the epinephrine infusion, plasma IRI suppression by epinephrine is clearly not sluggish in obesity. An inactive alpha receptor mechanism, therefore, does not contribute to the hyperinsulinism of obesity.

The 6 non-obese subjects were studied a second time after premedication for 4 days with 30 mg prednisone to determine if the hyperinsulinism in obese subjects could be simulated in non-obese subjects by administration of an insulin antagonist. After the non-obese subjects had been pretreated with prednisone, plasma IRI values were higher during the course of the experiment (Fig. 2). The difference was significant only at the fasting (F_1) sample and at 10 minutes during the infusion ($P<.05$). Considerably more variation in IRI concentrations was observed in the prednisone-treated subjects than in the other 2 groups. Suppression of IRI values during the epinephrine infusion was less in

TABLE I. Blood Glucose (mg %) in All Subject Groups Before and After Glucose Administration.*

Subjects	During epinephrine infusion (5 μg per min)						Post-infusion		
	F1	F2	5	10	15	20	5	10	15
Non-obese (6)	76 ± 2.6	78 ± 2.2	228 ± 6.0	216 ± 7.4	201 ± 7.3	195 ± 5.9	186 ± 7.2	182 ± 7.8	173 ± 7.6
Obese (7)	81 ± 3.4	78 ± 4.0	202 ± 4.2	196 ± 5.5	193 ± 4.2	189 ± 4.3	187 ± 6.4	181 ± 6.4	175 ± 8.2
Non-obese with prednisone (6)	80 ± 2.7	77 ± 2.4	204 ± 8.3	202 ± 8.2	197 ± 9.1	195 ±10.5	192 ±10.3	189 ±11.2	183 ±10.3

* Glucose (25 g) was administered intravenously immediately after the second blood sample (F2) had been obtained. Epinephrine infusion was begun immediately after F1 sample had been obtained.
Means ± S.E.M. are shown.
Numbers of subjects in parentheses.

prednisone-treated non-obese subjects than in untreated non-obese or obese subjects. The mean post-infusion IRI concentration was only 190% of the mean value during infusion (comparable values of 291 and 374% in the untreated non-obese and obese subjects respectively).

Blood glucose values for the 3 groups of experimental subjects are shown in Table I. The comparable fall in blood glucose in non-obese and obese subjects despite hyperinsulinism in the latter underscores the role of insulin resistance in obesity.

Summary. Obesity is characterized by fasting hyperinsulinism and elevated IRI concentrations during and following an epinephrine infusion. Suppression of plasma IRI by epinephrine is normal in obese, nondiabetic subjects. The concept of sluggish alpha receptors cannot be invoked as a cause of the hyperinsulinism of obesity.

1. Kreisberg, R. A., Boshell, B. R., DiPlacido, J., Roddman, R. F., New Engl. J. Med., 1967, v276, 314.
2. Karam, J. H., Grodsky, G. M., Forsham, P. H., Diabetes, 1963, v12, 197.
3. Beck, P., Koumans, J. H. T., Winterling, C. A., Stein, M. F., Daughaday, W. H., J. Lab. & Clin. Med., 1964, v64, 654.
4. Porte, D., J. Clin. Invest., 1967, v46, 86.
5. Klein, R. F., Troyer, W. G., Back, K. W., Hood, T. C., Bogdonoff, M. D., Metabolism, 1965, v14, 17.
6. Halpern, S. L., Gleen, M. B., Goodhart, R. S., J.A.M.A., 1960, v173, 1576.
7. Saifer, A., Gerstenfeld, S., J. Lab. & Clin. Med., 1958, v51, 448.
8. Genuth, S., Frohman, L. A., Lebovitz, H. E., J. Clin. Endocrinol., 1965, v25, 1043.

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Interferon and Murine Leukemia. — IV. Futher Studies on the Efficacy of Interferon Preparations Administered after Inoculation of Friend Virus. (32572)

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It has been reported previously that continued interferon treatment initiated 48 hours *after* inoculation of Friend virus inhibited the development of splenomegaly in Swiss mice (1). Although an increased number of "Friend cells" may be present in the spleen at this time (48 hours), significant splenic enlargement is not observed(2,3). It was considered of interest therefore to determine the effect of interferon on the evolution of Friend disease when treatment was initiated at an even later stage. Thus, in one of the experiments reported herein, interferon treatment was begun one week after viral inoculation, at a time when splenomegaly had already developed in most mice. In addition, we present the results of investigations pertaining to the mechanism and specificity of interferon action in Friend disease. These studies include histologic examinations of the spleens of interferon treated and untreated mice, assays of several of these spleens for infective virus, and the determination of the efficacy of a highly purified preparation of interferon.

Methods and materials. The techniques employed in the assay of Friend virus; the preparation of concentrated crude mouse brain interferon and its assay have been previously described in detail(4). They may be summarized briefly as follows: 4- to 6-week-old Swiss mice were inoculated intraperitoneally (i.p.) with a 10-15% extract of Friend leukemic spleen and sacrificed 2-4 weeks later. Spleen weights provided criteria for infection and extent of disease(5). Interferon was extracted from the brains of mice infected

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