place after dialysis and drying is shown by the fact that dried IF was not completely soluble when an attempt was made to redissolve it. It is noteworthy that IF clinically active at 1 mg was heterogeneous. Unless there is more than one substance that can act as IF, this would indicate that pure IF is physiologically active at less than 1 mg.

Summary. A highly purified intrinsic factor preparation (RAS fraction) was prepared in quantity from fresh hog duodenum by saline extraction, pH adjustment and ammonium sulfate precipitation. This fraction was active in pernicious anemia patients in relapse at daily oral dose of 5 mg. Ultracentrifugal separation could not completely separate the components of RAS fraction. RAS fraction was further purified on a DEAE-cellulose ion exchanger to give a fraction which was active in urinary excretion test at a dose of 3 mg, equivalent to a daily oral dose of 1 mg in pernicious anemia patients in relapse. Rechromatography of this active fraction resulted in no further increase in potency. Ultracentrifugal studies of the most active fraction obtained from rechromatography showed essentially 3 peaks with sedimentation constants of 1.0 S, 2.8 S and 11 S.

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Effect of 17-Ethyl-19-Nortestosterone on Hyperglycemic Action of Glucagon in Humans. (23873)

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There are only a few known conditions in which human subjects will fail to respond to intravenous injection of pancreatic hyperglycemic factor (HGF or glucagon) with substantial rise in blood sugar. These are: a) if liver glycogen is low, as in starvation, severe liver disease or diabetic acidosis, and b) if liver glycogen is abundant but a specific enzyme deficiency prevents glycogenolysis, *e.g.* deficiency of glucose - 6 - phosphatase in glycogen-storage disease(1).

A chance observation in one patient led to the suspicion that 17 ethyl-19-nortestosterone (Nilevar†) might depress glucagon-in-

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† Kindly supplied by Dr. Robert L. Craig, G. D. Searle and Co., Chicago, Ill.

duced hyperglycemia. Investigation of the effect of Nilevar on hyperglycemic response to glucagon was thus undertaken.

Methods. A total of 16 patients was studied. Eight were being treated with Nilevar when the study was instituted. At least one glucagon tolerance test was done on each patient while under therapy. When possible one or more tests were done at varying intervals after therapy had been stopped. The other 8 patients were treated with Nilevar after control glucagon tests were done. Glucagon response was then studied at repeated intervals while treatment continued. Most patients received Nilevar for relief of osteoporosis associated with some chronic illness, or because an anabolic hormone was indicated. Their nutritional status and daily food intake were fair to good. There were 2 males and 14 females with age range of 41-80 years. Three women were diabetics. Usual dose of Nilevar was 10 mg 3 times a day by mouth. In 2 patients this was later doubled, and 2 patients were given twice this dose from the beginning. One patient received only 10 mg twice a day. Glucagon tolerance test was done as standardized in this institution by several hundred tests. After 14-17 hour fast, 2 control blood specimens were taken at 10-minute intervals. 0.1 mg crystalline glucagon‡ was injected intravenously and blood specimens drawn 10, 20, 30, 45, 60, 90 and 120 minutes later. All were 0.1 ml samples taken in duplicate from finger blood. Blood sugar was determined by micro method of Somogyi as modified by Nelson(2). In 4 instances, patients were tested with 2 or 5 times the usual dose of glucagon. Twenty-four hour thyroid I^{131} uptakes were done on 8 patients while on Nilevar therapy.

Results. Table I summarizes results in the 16 patients. Nilevar produced a highly significant uniform depression of the hyperglycemic response to glucagon. Suppression of response to glucagon was complete in all patients except 3 (R.G., K.L. and L.V.) who received the drug only 8 days or less. From a large experience with this test, the lower limit of normal response to 0.1 mg glucagon given intravenously is a rise in blood sugar of 20 mg %. A rise of 15 mg % or less is considered a negative or absent response, 16-19 mg % being borderline.§

TABLE I. Maximum Blood Sugar Response to 0.1 mg H.G.F. Intravenously (Increase in Blood Sugar in mg %).

Patient	Off Nilevar	On Nilevar	Duration of Nilevar therapy at time of test
R.B.	33	13	9 days
R.B.	108	8	71 "
L.D.	70	0	67 "
E.E.		7	2 mo
R.G.	71	41	4 days
D.G.		11	3 mo
I.K.		5	2 "
J.K.	81	0	28 days
K.L.	57	20	8 "
I.P.	50	0	4 mo
B.P.	36	14	7 days
E.R.	29	7	2½ mo
L.V.		26	2 days
I.W.	101	12	17 "
F.W.	70	14	8 "
R.W.		0	34 "
*	64.2 ± 26.4	11.1 ± 10.9	
P		<.003	

* Mean ± S.D.

On a dose of 30 mg/day, beginning suppression was evident as early as 4 days in some patients. Suppression was complete by 9 days to one month in patients tested repeatedly. In 2 instances (R.B. and L.D.), the dose had to be doubled to 60 mg/day before negative tests were obtained. The 2 patients (B.P. and P.W.) given 60 mg/day initially showed absent glucagon responses more rapidly, *i.e.*, in 7 and 8 days respectively. 0.2 mg and 0.5 mg glucagon i.v. failed to change negative glucagon responses to positive ones.

Zero responses indicate no rise at all. Instead, blood sugars went below initial levels as much as 18 to 33 mg %. For statistical purposes, it was considered more valid and more significant to call these "zero" rather than "minus" responses. Had the latter been done, the difference between means would have been greater.

Table II demonstrates that mean response of Nilevar treated patients to glucagon is significantly different from that of previous large

‡ Kindly supplied by Dr. W. R. Kirtley, Lilly Research Laboratories, Indianapolis, Ind. Lot No. 0357-P-6046.

§ From unpublished data.

TABLE II. Maximum Blood Sugar Response to 0.1 mg H.G.F. Intravenously (Increase in Blood Sugar in mg %).

Condition	No. of patients	Mean	S.D.	P
Control*	37	51.4	12.8	
On Nilevar†	16	11.1	10.9	<.003

* Previous study. † This study.

control series of similar patients.

Fig. 1 demonstrates some typical curves of response to 0.1 mg HGF intravenously. As seen in Ic, the glucagon response returns toward normal shortly after Nilevar is discontinued.

The 24-hour thyroid I^{131} uptakes varied from 21-55%, only one being outside normal range, which for this laboratory is 15-50%.

The control of diabetic state was not obviously altered by Nilevar therapy in the 3 patients under observation. There was no evidence of hypoglycemia in non-diabetic subjects.

Discussion. It is apparent that Nilevar inhibits normal hyperglycemic response to glucagon. How this occurs is speculation. The first consideration is that Nilevar is a potent protein anabolic substance(3). Glucagon, on the other hand, has been shown recently to increase gluconeogenesis and to be protein-catabolic in a manner similar to, but independent of adrenal glucocorticoids(4). Does Nilevar block glucagon-induced hyperglycemia by preventing the enhanced gluconeogenesis and protein catabolism? If so, this would indicate that under certain circumstances, gluconeogenesis may be a more important mechanism of glucagon-caused hyperglycemia than liver glycogenolysis.

The fact that hyperglycemic response to glucagon is completely abolished only after many days or weeks of Nilevar therapy suggests liver glycogen depletion. Pretreatment with adrenocorticotrophic hormone (ACTH), cortisone or somatotrophic hormone (STH) enhance the hyperglycemic response to glucagon, presumably because they increase liver glycogen deposition(1). Since Nilevar has progestational and androgenic activity(3,5), it may inhibit pituitary and hence adrenal gluco-corticoid secretion. This might result in decreased liver glycogen and diminished hyperglycemic response to glucagon, which

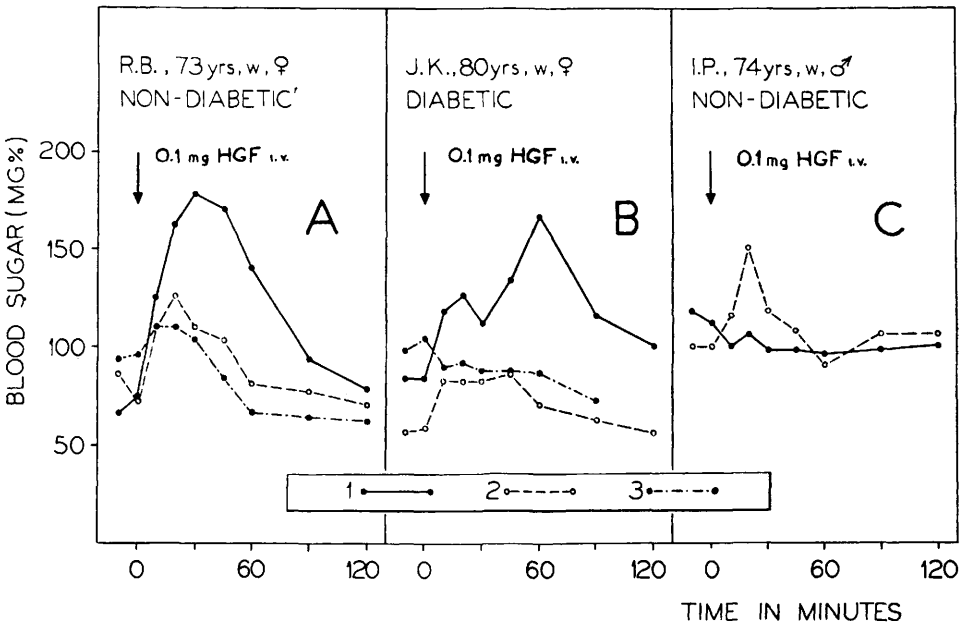

FIG. 1. Typical curves of response of blood sugar to 0.1 mg HGF intrav. before and during Nilevar therapy. A. Curve (1) before Nilevar; (2) 30 mg Nilevar/day for 12 days; (3) 30 mg Nilevar/day for 56 days and 60 mg/day for 6 more days. B. Curve (1) 30 mg Nilevar/day for 1 day; (2) 30 mg Nilevar/day for 12 days; (3) 30 mg Nilevar/day for 28 days. C. Curve (1) 30 mg Nilevar/day for 4 mo; (2) 12 days after Nilevar discontinued.

acts primarily by increasing liver glycogenolysis(1). Unpublished data on 5 patients in this hospital‖ demonstrate that Nilevar therapy does not alter plasma gluco-corticoid levels or ACTH responsiveness of adrenal glands. The normal I^{131} uptakes of thyroids of patients in our study are also indirect evidence of lack of pituitary inhibition by this drug. However, this does not rule out the possibility of direct interference by Nilevar of gluco-corticoid or other hormonal enhancement of liver glycogen deposition.

Both mechanisms may contribute to our findings, *i.e.,* diminished liver glycogen and direct antagonism of anabolic and catabolic substance. Nilevar, by decreasing gluconeogenesis by glucagon or adrenal glucocorticoids may gradually deplete the liver of glycogen. There is the possibility that Nilevar is an enzyme poison or inhibitor. This is of course compatible with any of the above mentioned theoretical mechanisms. Finally, Nilevar may produce this alteration of liver function by causing actual liver cell damage. The rapid return to a normal glucagon response in all patients after Nilevar was discontinued, argues against this.

Whatever the mechanism(s) may be, there exists the intriguing thought that therein may lie a clue to the role of glucagon in normal body economy.

Summary. Sixteen patients were studied as to the effect of Nilevar on hyperglycemic response to glucagon. Nilevar abolished or profoundly diminished this response. This effect was reversible when Nilevar therapy was stopped. Nilevar had no apparent effect on the control of 3 diabetic patients nor on the 24-hour thyroid I^{131} uptakes of 8 patients. Possible mechanisms are discussed.

‖ Studied in collaboration with Dr. M. G. Goldner and Dr. A. Carter.

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Strain Gauge Measurement of Tension Changes in Rat Colon.* (23874)

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In both rat and dog, we have found a direct association between blood pressure changes and movements of sodium into and out of extracellular compartment(1,2). Our current interpretation of these and other results is that blood pressure regulation depends on sodium transfer systems, broadly defined, which govern rate of sodium exchange between vascular smooth muscle and the surrounding medium. Definitive proof of this theory depends on study of the response of smooth muscle (vascular and other) to acute alterations of the cationic medium, or on analysis of cationic changes in the medium during and after induction of contraction in the muscle strip.

Methods currently in use for study of smooth muscle *in vitro* are unfortunately not applicable to this problem, since they would not permit rapid analysis or rapid alteration of the medium and further, the high volume of the medium relative to tissue would tend to dilute sodium shifts beyond detection limits of current methods. Thus, we have prepared new instruments. These have turned out to be simple, rugged and highly sensitive; further, they seem to us to be generally useful for study of smooth muscle strips. The present

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