To speculate briefly on the purpose served by this inhibitory factor, it may be pointed out that the estrus cycle of the rat is so short that the closely repeated stimulations of the uterus by estrin might lead to a chronic state of estrus, were it not for the presence of a mechanism which sharply limits the response of the uterus to this hormone, at the appropriate time in the cycle.

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Activity of Insulin in Diabetic Hyperglycemic Animals.

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Hyperglycemia is a constant accompaniment of severe untreated diabetes mellitus. Does it of itself interfere with the ability of the organism to utilize carbohydrate? Previous to the use of insulin, a fall in the blood sugar and a decrease in the severity of the diabetes was noted with fasting.[1, 2] With the use of insulin the control of hyperglycemia is accompanied by a decrease in the daily insulin requirement of patients.[3] In view of the fact that in both of these conditions the prevailing blood sugar level is lowered, the following question naturally presents itself: Does the hyperglycemia *per se* interfere with the ordinary action of insulin?

The activity of insulin at normal blood sugar levels was studied by Greeley.[4] He found that with a certain hourly intravenous dose of insulin, the blood sugar of a diabetic animal could be maintained at a normal level without change. For the individual fasting, depancreatized dog, this dose was remarkably constant over a long period of time and was consequently termed the "Basal Insulin Requirement" and expressed in units of insulin per kilogram of body weight per hour. In the present work this "Basal Insulin Requirement" was first determined on the experimental dog (at a normal blood sugar level). Then on other experimental days the animals was given insulin at this rate (or less) when the blood sugar was at a hyperglycemic level. The essential difference in procedures is

1 Allen, F. M., *J. Am. Med. Assn.*, 1914, **63**, 939.

2 Newburgh, L. H., and Marsh, P. L., *Arch. Int. Med.*, 1920, **26**, 647; 1921, **27**, 699.

3 Joslin, E. P., *Treatment of Diabetes*, Ed. 6, 305, Lea and Febiger, Phil., 1937.

4 Greeley, P. O., *Am. J. Physiol.*, 1937, **120**, 345.

that in determining the basal requirement the blood sugar level is first brought to normal levels by means of extra insulin.

Experiments were carried out on 8 depancreatized dogs which had been maintained with insulin* on a constant diet until they had recovered from the operation. Prior to the experimental periods the animals were fasted at least 24 hours and the insulin withheld 12 to 24 hours. The basal insulin requirement of each animal was determined during control periods. At other periods and when the animals were hyperglycemic, insulin was given intravenously at hourly intervals in amounts ranging from 42 to 100% of the basal requirement and the effect on the blood sugar observed. The results are reported in Table I. In 2 of these experiments the presence of sugar in the urine was determined at times when the blood sugar had ceased to change.

TABLE I.

Records of blood sugars on individual dogs for periods during which insulin was given at the hourly rates indicated at the top of the columns opposite the heading "Sub-basal Dose." The figures opposite "Basal Dose" indicate the amount of insulin that has to be given per hour to maintain the blood sugar at a constant normal level (around 100 mg.%); this determination was made during control experimental runs.

Dog	Br	Bi	M	W	J	Th	E	K
Sex	Female	Female	Male	Male	Male	Female	Female	Male
Weight, kg.	7.7	6.5	6.8	6.5	7.1	6.1	14.8	7.3
Basal dose, u./kg./hr.	.019	.023	.061	.038	.01	.037	.041	.01
Sub-basal dose, u./kg./hr.	.008	.020	.029	.020	.01	.016	.027	.01
% basal dose	42	87	47	53	100	43	65	100
Hours			Blood Sugar mg.%.					
0	320	364	384	400	276	286	482	440
1	254	—	332	284	284	276	400	—
2	222	—	—	266	250	222	—	382
3	200	335	346	—	264	206	380	—
4	190	—	336‡	276	240	186	300	286
5	182	284	312	242	250	178	346	—
6	166	—	320	266	228	182	328	206
7	166	—	286	260	234	178	306	196
8	190*	196	258§	276	224	—	298	185
9	198	—	236	266	234	180	266	182
10	182	163	222§	263	222	176	258	186
11	184	—	—	258	228	178	243	—
12	174†	—	—	265	216	174	242	—
13	174	167	—	—	—	—	—	—
14	—	168	—	—	—	—	—	—
15	—	165	—	—	—	—	—	—

*Catheterized and bladder washed.
†Catheterized: Urine sugar: trace.
‡Urinated: Urine discarded.
§Urine collected: Urine sugar: trace.

* Appreciation is expressed for a grant of Insulin (Iletin) by Eli Lilly and Co.

Additional experiments were carried out on Dog Br in which different sub-basal doses of insulin were given at hyperglycemic levels. Fig. 1 shows the control period, that is the maintenance of the normal blood sugar level by 0.020 u./kg./hr. after the blood sugar level had been brought to a normal level by higher doses of 0.040 u./kg./hr. The administration of 0.004 u./kg./hr. (26% of basal requirement) resulted in a fall from 320 to 218 mg. % at the seventh hour, after which the level remained between 182 and 230 mg. % until the thirteenth hour. At another time (not shown in the chart) 0.013 u./kg./hr. (68% of basal insulin requirement) was given and the blood sugar fell from 348 to 202 mg. % at the fourth hour, remaining between 182 and 210 mg. % until the tenth hour.

During an experiment on Dog M, 0.059 u./kg./hr. (97% of basal requirement) was accompanied by a drop from 408 to 181 mg. % at the fourth hour. Then insulin was given in amounts over the basal requirement (fifth hour: 0.090 u./kg./hr., 148%); and at the sixth and seventh hours: 0.118 u./kg./hr. (177%). At the eighth hour the blood sugar was 118 mg. % and the hourly dose of 0.059 u./kg. was again administered, the blood sugar rising to 125 mg. % at the eleventh hour.

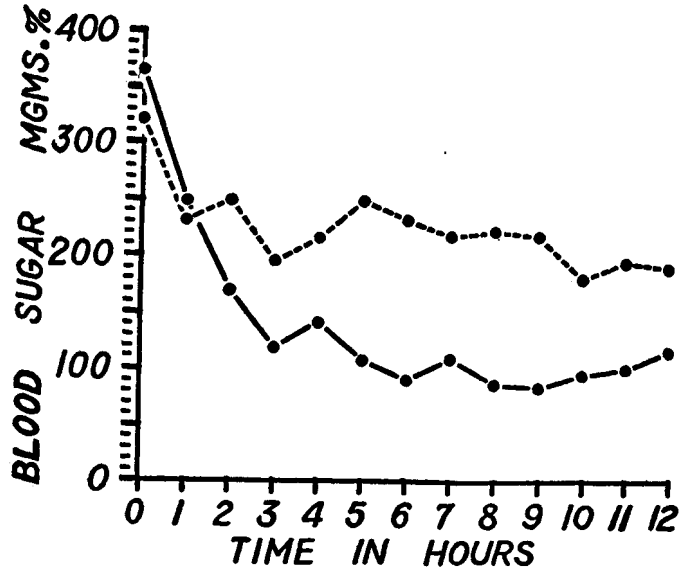

FIG. 1.

Records of blood sugars on the same animal in two separate experiments. Exp. 1 (Continuous line) blood sugar brought to normal region (120 mg.%) by injection of 0.4 u./kg./hr. insulin during the first two hours, thereafter the level is maintained by the injection of 0.02 u./kg./hr. insulin. Exp. 2 (Broken line) blood sugar course with hourly injections of 0.004 u./kg./hr. insulin.

In all cases there was a fall in the blood sugar level when insulin was injected into hyperglycemic animals at sub-basal rates. The blood sugar rose from normal levels if these amounts were given during the control periods. The rapidity of decrease in hyperglycemia varied in different animals and was not related to the percentage of the basal insulin requirement given. After a period of 2 to 11 hours, the levels remained constant for the remainder of the experimental periods. The blood sugar concentration at which these constant levels appeared, varied from 163 to 276 mg. %, and in experiments on the Dog Br did not appear to be closely related to the different percentages of the basal requirement which were given. An explanation of the fall in the blood sugar levels with sub-basal hourly doses of insulin is that kidney excretion disposes of that portion of new sugar formation of the organism which is not utilized with insulin. Variation in the rapidity of the fall results not only from differences in the amounts of sugar utilized or produced in the presence of insulin, but the actual speed with which the kidney can excrete glucose in the urine. As the falling blood sugar level approaches the kidney threshold for glucose, the amount of sugar excreted in the urine tends to equal the quantity of unutilized sugar, so that the blood sugar level ceases to change. That the activity of insulin is at least as great at these constant levels as at normal levels is shown by the results in experiments where the urinary sugar was determined and only a trace was found. Indeed it would seem that the insulin might have a hyperactivity at higher blood sugar levels.

Conclusions. 1. In 8 diabetic dogs there was a definite decrease in the hyperglycemia during the hourly injection of amounts of insulin less than that required for the maintenance of a constant blood sugar level within the normal physiological range. Hourly amounts of insulin which would have allowed the blood sugar to rise when given at a level of 100 mg. % caused the blood sugar to fall when given at hyperglycemic levels. 2. In untreated diabetes mellitus, factors other than the hyperglycemia must be responsible for the severity of the condition, as the high blood sugar *per se* does not interfere with the ordinary action of insulin.