caproate as a substrate. At the same time there was a 15-100 fold greater incorporation of C^{14} bicarbonate into the carboxyl group of acetoacetate produced by homogenates of livers from biotin repleted rats than by the deficient ones.

With the homogenate systems employed here, isovalerate leads to a marked increase in the radioactivity of the carboxyl group of acetoacetate, though no significant net formation of ketone bodies can be detected(3). However, biotin treatment of the deficient rats resulted in a 12-fold increase of C^{14} incorporation into acetoacetate derived from isovalerate.

The decreased *incorporation* of C^{14} from bicarbonate into the carboxyl group of acetoacetate by rat liver homogenates from biotin deficient animals as compared to those from the repleted ones seems to be the result of an effect on the bicarbonate incorporating mechanism, since the *amount* of this keto acid produced by the liver homogenates from a number of substrates was not influenced.

Summary. The quantity of acetoacetic acid produced metabolically from pyruvate, caprylate, and caproate by rat liver homogenate systems is not affected by the state of biotin nutrition of the animals. However, biotin treatment of deficient animals increased by 10-100 fold the incorporation of C^{14} bicarbonate into the carboxyl group of acetoacetate metabolically formed from pyruvate, caproate, caprylate or isovalerate.

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Blood Glucose Levels in Alloxan-Diabetic Rats under Combined Insulin and Ergot Alkaloid Therapy.*† (19213)

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Dubois, Herrmann, and Erway(1) have reported that the hyperglycemia produced by the rodenticide, alpha-napthylthiourea (ANTU), is conteracted by ergotamine, which is a natural alkaloid of ergot, as well as by insulin. They accounted for this parallelism through assumption that there are antagonistic actions of both ergotamine and insulin toward the glycogenolytic action of epinephrine, the production of which is stimulated by the rodenticide. Stoll and Hofmann(2) have been responsible for the production of dihydrogenated derivatives of the ergotoxine group of ergot alkaloids. It has been shown by Rothlin(3) that the hyperglycemia produced in animals through administration of epinephrine is inhibited by the hydrogenated ergot alkaloids. Freis, Stanton, and Wilkins(4) have demonstrated adrenolytic activity of dihydroergocornine (DHO 180) and dihydroergokryptine (DHK 135) in humans and have also shown, under proper experimental conditions, that the pressor response, tachycardia, and mydriasis which normally follow epinephrine administration are reduced by these preparations. Schneider(5) found that the period of hypoglycemia in humans, produced by the intravenous injection of small doses of insulin, was not lengthened by administration of dihydroergocristine, dihydroergocornine, or

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dihydroergokryptine. It has been shown by Nitsch(6) that Hydergine (CCK 179) which is a mixture of dihydroergocornine, dihydroergocristine, and dihydroergokryptine, injected before administration of insulin, served to reduce oscillations of blood glucose to a considerable degree when such oscillations were fairly marked but that the preparation had little or no tendency to potentiate the action of insulin in those cases with only slight blood glucose oscillations.

The present investigation was originally designed to determine whether the insulin regulation of blood glucose levels would be interfered with by the administration of the dihydrogenated derivatives of ergot. The effects upon the blood glucose levels of insulin-treated alloxan-diabetic rats resulting from administration of ergotamine tartrate, dihydroergocornine, and Hydergine are compared.

Materials and methods. Albino rats of the Sprague-Dawley strain and of both sexes were used in this investigation. All animals were between 100 and 150 days of age and all were fed a stock diet commercially manufactured and containing the usual proportions of protein, fat and carbohydrate; drinking water was supplied *ad libitum*. The induction of alloxan diabetes was accomplished through intraperitoneal injection of alloxan monohydrate (approximately 150 mg per kg of body weight) dissolved in distilled water as described by Gomori and Goldner(7). It was considered that a diabetic state existed when the blood glucose level reached or exceeded 200 mg %. As has been previously shown by the report of Sinden and Longwell(8), it was found that the amount of protamine zinc insulin necessary to maintain satisfactory blood glucose levels was more or less arbitrary. In general, the following amounts were found to be fairly satisfactory: 7 units protamine zinc insulin per 24 hours if the blood glucose in mg % was over 500; 5 units protamine zinc insulin per 24 hours if the blood glucose level in mg % ranged between 250 and 500: 3 units protamine zinc insulin per 24 hours if the blood glucose level in mg % ranged between 200 and 250. Insulin was injected subcutaneously at 24-hour intervals. Determinations of blood glucose levels were accomplished through removal of blood samples from the tips of the tails of the rats with heparin being used as an anti-coagulant. Blood glucose determinations were made according to the method of Folin and Wu(9) as modified by Fiorentino and Giannettasio(10). Blood glucose levels were determined in normal and alloxan-diabetic rats immediately prior to insulin administration and at intervals of 1, 3, 7, and 10 hours after it had been given. Accordingly, the first blood sample was obtained at 10:00 a.m., insulin was given immediately thereafter, and subsequent determinations of blood glucose levels were made at 11:00 a.m., 1:00 p.m., 5:00 p.m., and 8:00 p.m. When insulin administration was omitted blood glucose determinations were carried out at the same hours in order to provide accurate comparisons of blood glucose levels in insulin-controlled and uncontrolled alloxan-diabetes. To study the effects of combinations of protamine zinc insulin with Hydergine, dihydroergocornine or ergotamine tartrate, the ergot alkaloids were injected intraperitoneally 2½ hours after a regular 24-hour subcutaneous injection of insulin in the amount of 4 mg per kg of body weight. The time interval of 2½ hours between injection of insulin and that of the alkaloids was selected because it was found, once insulin control of the blood glucose level in the alloxan-diabetic rat had been established, that the period during which the blood glucose level remained fairly constant was between the third and tenth hours following the injection of insulin. Blood glucose levels under combined therapy were determined at the same intervals and at the same times of day as in the normal and alloxan-diabetic animals.

Results. The blood glucose determinations for each series of animals are presented in Table I. That diabetes was present in the alloxan-treated animals is evident when parts a and b of Table I are compared. The effectiveness of insulin in controlling the blood glucose levels of alloxan-diabetic rats is readily apparent when one compares part c of Table I with part b. Examination of Table I, d

TABLE I. Blood Glucose Determinations in mg % in Normal, Diabetic, and Diabetic Rats Under Therapy.

	10 a.m.	11 a.m.	1 p.m.	5 p.m.	8 p.m.
a. Normal, untreated	108	120	128	112	120
	112	130	120	116	116
	116	136	130	116	116
	110	115	112	116	118
	114	120	125	118	112
Avg	112	124	123	116	116
b. Diabetic, untreated	225	245	240	700	240
	425	375	440	375	345
	570	570	570	550	475
	335	340	370	350	335
	420	450	430	430	450
Avg	395	396	410	481	369
c. " insulin treated	76	56	46	42	104
	144	128	84	52	136
	124	112	64	124	116
	68	64	52	66	64
	156	76	84	30	64
	120	64	40	40	52
Avg	115	83	62	59	89
d. " " " + ergotamine	398	276	42	42	68
	140	120	112	52	116
	284	354	60	42	48
	372	364	184	90	34
	112	112	34	68	34
	316	316	140	120	76
	140	140	60	42	40
Avg	251	240	90	65	59
e. " " " + CCK 179	135	80	45	60	50
	140	325	45	45	50
	350	60	40	60	70
	80	60	60	50	55
	480	440	55	85	130
Avg	237	193	49	60	71
f. " " " + DHO 180	208	212	36	46	58
	120	64	42	52	68
	280	152	112	64	76
	60	58	52	38	64
	60	126	52	46	60
	48	64	36	42	58
	144	46	46	46	58
	360	152	60	108	64
	152	112	60	84	94
	184	94	52	84	94
Avg	162	108	55	61	69

indicates that the combination of ergotamine and insulin resulted in a more prolonged and somewhat more pronounced depression of blood glucose levels than was produced by insulin alone. The administration of Hydergine (CCK 179) or dihydroergocornine (DHO 180) with insulin, as indicated in parts e and f of Table I, did not appreciably change the blood glucose levels from those resulting from insulin alone as observed over a 10-hour period; as with insulin alone the hypoglycemia was beginning to moderate at the end of 10 hours and this compared with a downward trend at the same interval in the animals given insulin and ergotamine tartrate.

The average mg % change in blood glucose levels between the third and tenth hours after insulin injection and after insulin injection followed in 2½ hours by injection of one of the alkaloids was calculated. In those diabetic rats treated with insulin the average blood glucose level increased an average of 28 mg % between the third and tenth hours after insulin injection. When ergotamine was combined

with insulin, the average blood glucose level dropped 31 mg % during the same interval. Combinations of Hydergine and dihydroergocornine with insulin were associated respectively with rises of 22 mg % and 8 mg % during the interval between the third and tenth hours after injection of insulin. When the "t" test of the difference between means of small samples was applied, the difference between insulin-treated and insulin-plus-ergotamine-treated animals showed a probability ("P") of 0.05. The probabilities when Hydergine or dihydroergocornine were administered in conjunction with insulin, were respectively 0.75 and 0.2. The probability values indicate that the effects upon blood glucose levels resulting from combined administration of ergotamine and insulin are statistically more significant than those produced by combined administration of Hydergine or dihydroergocornine and insulin.

Discussion. Since adequate control of blood glucose levels is essential during treatment of diabetic gangrene, diabetic ulcers, or other complications of diabetes, it is important that any therapeutic agents employed in conjunction with insulin do not alter appreciably its effect upon blood glucose. It appears that Hydergine and dihydroergocornine have relatively less marked effects on the blood glucose level when combined with insulin therapy than is the case when insulin and ergotamine tartrate are combined.

With regard to the use of Hydergine or dihydroergocornine in the treatment of the circulatory complications of diabetes, it has been demonstrated that they produce peripheral vasodilatation(11) which is usually beneficial in the treatment of such complications. Rothlin(12) has stated that the hydrogenated alkaloids constituting Hydergine are the only known substances known up to now which have, simultaneously, the properties of: (a) dilating the vessels and lowering arterial tension by manifest action upon the vasomotor center; (b) preventing, by inhibition of the pressosensory reflexes, a compensating increase in arterial tension; (c) assuring, due to their central bradycardic effects, an adequate cardiac output by adaptation of the heart to the increase in peripheral flow.

Tests(13) have shown that the average lethal dose of Hydergine is 100 to 1000 times as great as its effective dosage. It seems likely, on the basis of such tests, and on the basis of the observations presented herein that either Hydergine or dihydroergocornine may be used in the diabetic patient without significantly disturbing blood glucose levels as maintained by insulin therapy.

Summary. A series of alloxan-diabetic rats under insulin therapy was treated with ergotamine tartrate, Hydergine (CCK 179) and dihydroergocornine (DHO 180). The following results are recorded: Ergotamine tartrate lowered the average blood glucose value during a period of 3-10 hours after the regular 24-hour insulin injection; dihydrogenated ergot alkaloids, (dihydroergocornine or Hydergine, which is a mixture of dihydroergocornine, dihydroergokryptine and dihydroergocristine) on the other hand, seem to have little effect on the blood glucose level as regulated by insulin therapy.

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