hours after injection the average bleeding volume in the thyroxin treated animals (1.67 cc) is significantly less than that of the normal animals (4.24 cc) and is only approximately 18% of the value obtained prior to the injection of ergotamine.

It appears that the effect of thyroxin in increasing the susceptibility of the rat to ergotamine-induced gangrene of the tail is essentially one of prolongation of the vascular occlusion produced by ergotamine. Such an effect may be due to sensitization of the vascular musculature to the action of ergot as has been claimed for epinephrine(14). It would seem that such should result in an increase in the intensity as well as the duration of the effect. However, the present method may be too indelicate to detect an increase in an already severe vasospasm. Alternatively, thyroxin may interfere with the destruction and/or elimination of the ergot alkaloids, thus, prolonging without intensifying the effect.

In partial support of this latter hypothesis is the observation that 8 out of 10 rats developed gangrene following the injection of 12.5 mg/kg of ergotamine subsequent to exposure to chloroform vapor (2 hours in a concentration of 1.77 vol. %). This is a significant ($p = <0.02$) increase in the incidence of gangrene over normal and suggests that liver damage, by interfering with the elimination of ergot alkaloids, may predispose to ergot gangrene.

Summary. Thyrotoxicosis increases the incidence of ergotamine-induced gangrene of the tail of the rat. Reduction in oxygen consumption by propylthiouracil does not alter the incidence of ergot gangrene. Bleeding from the amputated tail of the rat is markedly reduced for prolonged periods by ergotamine and pre-treatment with thyroxin further prolongs the vasospasm so induced. It is assumed that thyroxin acts, not by altering the resistance of the tissues to vascular occlusion, nor by sensitizing the vascular musculature to ergotamine, but by prolonging the action of the alkaloids, possibly by interfering with their elimination or destruction.

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Effect of Hyperglycemic-Glycogenolytic Factor on Blood Sugar of Patients with Diabetes. (17914)

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In a previous communication(1) the clinical and experimental evidence suggesting that a hyperglycemic substance obtained from the pancreas may be an alpha cell hormone which causes an elevation of blood sugar by promoting hepatic glycogenolysis was reviewed. As stressed by Weisberg *et al.*(2) thus far it has not been proved that this material functions physiologically nor that it is a true hormone. This substance has been designated by Sutherland and his co-workers(3-5) as a hyperglycemic-glycogenolytic factor or H-G F.

If the H-G factor is an alpha cell hormone, it was believed that certain patients might exhibit evidences of increased or decreased activity of this substance as suggested by Thorogood and Zimmerman(6), McQuarrie

1. Pincus, I. J., *J. Clin. Endocrin.*, 1950, v10, 556.

2. Weisberg, H. F., Caren, R., Huddleston, B., and Levine, R., *Am. J. Physiol.*, 1949, v159, 98.

3. Sutherland, E. W., and Cori, C. F., *J. Biol. Chem.*, 1948, v172, 737.

4. Sutherland, E. W., and de Duve, C., *J. Biol. Chem.*, 1948, v175, 636.

5. Sutherland, E. W., Cori, C. F., Haynes, R., and Olsen, N. S., *J. Biol. Chem.*, 1949, v180, 825.

et al.(7) and others. Furthermore the possibility was entertained that variations in the activity of this factor might account for certain differences noted in patients with clinical diabetes. If the patient with "stable diabetes" has normal amounts of alpha cell function the administration of insulin might, directly or indirectly, produce a secretion of the H-G F preventing hypoglycemia and possibly increasing the insulin requirement. Some patients with a labile form of diabetes might have inadequate amounts of the H-G F and would therefore be very sensitive to insulin and show greater fluctuations of blood sugar after food or insulin. Such a patient might also develop acidosis readily since it has been suggested(6) that the alpha cell hormone might prevent or decrease ketosis. Patients in this group might also respond more vigorously than the stable diabetic when the H-G factor is administered. Hildes, Sherlock and Walsh(8-9) studied hepatic glycogen stores in diabetic patients and found them to be somewhat low but within the range of normal values. They also measured the responsiveness of different patients to a constant infusion of adrenalin. They noted that certain patients with labile diabetes failed to show a normal rise of blood sugar and some of these same patients exhibited a rise in hepatic glycogen levels in contrast to the normal and the stable diabetic group. This might suggest that these individuals are unable to use their hepatic glycogen stores in a normal manner and that they may be lacking in some factor necessary for adequate glycogenolysis.

The intravenous injection of insulin for determining insulin sensitivity and in treating patients in diabetic coma has not been attended by any remarkable or unpredictable side effects. It was felt that insulin preparations treated so as to destroy the insulin content but permit the continued activity of the H-G factor, which is present as a contaminant, should have no deleterious effects. Material thus obtained was injected into mice subcutaneously and intraperitoneally. It has been administered intravenously to rats, rabbits and dogs repeatedly and in large amounts with no evidence of toxicity. Three patients with inoperable carcinoma and one infant with hydrocephalus likewise exhibited no deleterious effects after receiving this substance in amounts as great as 1.0 cc per 10 kg of body weight. In view of the absence of any permanent or lasting effects beyond the temporary elevation of blood sugar the study to be reported was undertaken.

Material. Patients with diabetes were divided into 2 classes insofar as possible. The clinical classification suggested by Himsworth(10) was used although the insulin-glucose test devised by that author was not employed. One group consisted of older diabetics with blood sugar levels and glycosuria controlled by diet alone or in conjunction with varying amounts of insulin, in some instances as much as 100 units daily. These patients had rare episodes of hypoglycemia or acidosis, tended to be obese or normal in weight and for our purposes shall be referred to as "stable diabetics." A second group of diabetic patients were either young or had, with one exception (case No. 22), developed diabetes in early life. Their insulin requirement varied but in no instance was as high as some of the patients in the first group; they had experienced repeated episodes of hypoglycemia and of acidosis, even coma in some cases. These patients were thin or of normal weight. Control of the diabetes in this group was difficult to maintain. We shall consider these as "labile diabetics." A group of patients with diabetes which we were unable to classify,* with hepatic disease and a few patients with miscellaneous conditions were studied, as were a few patients convalescing from various diseases not involving disturbances of the liver or of carbohydrate metabolism.

6. Thorogood, E., and Zimmermann, B., *Endocrinology,* 1945, v37, 191.

7. McQuarrie, I., Bell, E. T., Zimmermann, B., and Wright, W. S., *Fed. Proc.,* 1950, v9, 337.

8. Hildes, J. A., Sherlock, S., and Walshe, V., *Clin. Sci.,* 1948, v7, 287.

9. Hildes, J. A., Sherlock, S., and Walshe, V., *Clin. Sci.,* 1948, v7, 297.

10. Himsworth, H. P., and Kerr, R. B., *Clin. Sci.,* 1939, v4, 119.

Methods. Two lots of Insulin, Squibb (No. 886 and 888) containing 80 units per cc and known to have relatively large amounts of the H-G factor were treated by heat and alkali as described by Sutherland(3). To 10 cc of the insulin preparation were added 0.8 cc of normal sodium hydroxide. This mixture was then incubated for 3 hours at 37°C. At the end of this time 0.8 cc of normal hydrochloric acid was added. The material was cultured for sterility and 1.0 cc injected subcutaneously into a mouse, to test for residual insulin. Patients were tested by injecting intravenously 0.5 cc of the "inactivated insulin" preparation per 10 kg of body weight. Some tests were performed in the fasting state in the early morning and others in the late morning after the patient had received breakfast and the usual morning dose of insulin. Blood sugar determinations were made by the method of Folin and Wu (11) on specimens taken prior to the injection and at 15 and 30 minute intervals. In a number of cases, as well as in many experimental observations in animals, specimens were also obtained at 5, 45, 60, 90, and 120 minutes. Little added information was thus obtained. In a few of these cases adrenalin tolerance tests were performed as described by Kinsell(12). These tests were performed on the same patients as were the tests with the H-G factor and under conditions as nearly identical as possible with those employed in the former test. Adrenalin chloride was injected subcutaneously, 0.1 cc of a 1:1000 solution per 10 kg of body weight being administered. Blood specimens were obtained prior to injection and at 30, 45, and 60 minute intervals for sugar determination.

Results. Systemic reactions consisting of urticaria, erythema or pruritis occurred in 3 patients, 2 of whom had in the past received insulin but did not require it when the test was performed. In 1 additional case a strongly positive skin reaction precluded the performance of the test. The reactions responded readily to antihistaminic preparations and adrenalin. Repeated tests performed on 3 patients before breakfast and in the late morning with different initial sugar levels in 2 showed a rise of the same magnitude whether the initial level was high or relatively normal. Therefore, the actual rise in blood sugar in milligrams per 100 cc has been presented rather than the percentile change. Experimental observations in animals also suggested that the initial blood sugar levels did not affect the elevation of blood sugar. The results obtained in this group of studies are presented in Table I. It will be noted that a fall in blood sugar occurred in several instances. We are not able to account for these cases but minimal amounts of insulin may have been present in some preparations. The normal patients and patients with a stable form of diabetes showed rises of blood sugar which were quite comparable, averaging 13.2 and 17.5 mg per 100 cc respectively with a maximum rise in this group of 25 mg per 100 cc. Among the labile diabetics, 6 tests performed in 5 patients produced an elevation of 41 to 108 mg per 100 cc. A sixth patient (Case No. 23) with diabetes which must be classified as labile had mild hypoglycemic symptoms and low blood sugar levels. When tested, at noon, with both adrenalin and the H-G factor she failed to respond to either of these agents. The average rise for this entire group was 57.7 mg† per 100 cc.

Too few adrenalin tests were performed to draw any conclusions. There was no evident correlation, however, between the rise of the blood sugar in any individual case

* Case No. 24, 25, 26 have at times required or at least tolerated large doses of insulin, (80, 150, and 65 units daily) at other times have been well maintained on no insulin. Case 27 is a patient with diabetes and recent Addison's disease. Case 28 has had repeated episodes of pancreatitis with calcification of the pancreas, steatorrhea and diabetes requiring 15 units of insulin daily in the past.

11. Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, v41, 367.

12. Kinsell, L. W., Michaels, G. D., Weiss, H. A., and Barton, H. C., *Am. J. Med. Sci.*, 1949, v217, 554.

† Statistical analysis of these data reveals a "t" value of 13.5. From the probability tables it would appear that the possibility of these results occurring as a matter of chance would be considerably less than 1%.

TABLE I.
Effect of Intravenous Injection of Hyperglycemic-glycogenolytic Factor and Subcutaneous Injection of Adrenalin on Blood Sugar Levels of Patients.

Case No.	Sex	Age, yrs	Wt., lb.	Insulin, U/day	Hyperglycemic factor from inact. insulin: Initial bl. sugar mg %	Max. change bl. s., mg %	Time, min.	Adrenalin: Initial bl. sugar, mg %	Max. change bl. s., mg %	Time, min.
					Normal					
1	M	21	140	0	95	21	(15)	98	77	(45)
2	M	23	152	0	97	15	(15)	94	83	(45)
3	M	28	145	0	84	19	(15)			
4	M	24	160	0	80	20	(15)			
5	M	31	168	0	121	—9	(30)			
					Stable diabetics					
6	M	42	151	25	100	14	(5)			
7	M	58	170	15	133	10	(15)			
8	M	49	185	20	139	19	(15)			
9	M	46	125	24	272	26	(30)			
9				20	141	20	(15)			
10	M	61	185	30	121	24	(15)			
11	M	49	190	15	80	20	(15)			
12	F	38	150	55	226	22	(15)	232	17	(45)
13	M	48	175	0	99	25	(30)	102	98	(60)
14	F	45	161	30	177	22	(15)	248	—14	(45)
15	F	58	218	100	356	18	(15)			
16	F	42	243	105	324	—8	(15)			
17*	F	65	154	35	300	16	(15)			
					Labile diabetics					
18	M	18	127	40	225	42	(15)			
19	M	54	160	40	140	60	(45)			
19		55	158	56	250	75	(30)	179	169	(60)
20	M	19	162	75	127	41	(15)			
21	M	19	133	60	266	108	(15)	324	22	(30)
22	F	50	115	35	438	74	(30)	330	42	(45)
23	F	22	129	52	40	4	(15)	58	3	(30)
					Unclassified diabetics					
24	M	24	145	0	232	3	(15)			
25*	F	46	166	0	114	10	(15)	118	49	(60)
26	M	54	137	0	62	22	(15)			
27	F	48	137	0	119	26	(15)	98	35	(45)
28*	M	39	132	0	127	31	(15)	167	95	(60)
					Hepatic diseases					
29	F	42	122	0	204	—75	(60)			
30	M	49	142	0	91	43	(15)	94	92	(45)
31	F	42	137	0	80	15	(15)			
32	F	50	177	0	110	6	(15)			
32		51	180	0	97	13	(15)	87	63	(45)
					Miscellaneous					
33	F	2.5	32	0	23	11	(15)	30	8	(45)
34	M	48	170	0	99	14	(15)			

* Reaction occurred soon after the injection of the HGF.

in response to either the H-G factor or adrenalin.

Comment. It would seem that if the labile diabetic is deficient in alpha cell function, a certain amount of glycogen deposited in the liver is not used as adequately as in the normal individual. When the H-G factor is administered, even in small amounts, this liver glycogen is mobilized. Lukens(13) suggested that the H-G factor, as it is con-

cerned in carbohydrate metabolism assists, rather than opposes, the action of insulin since it makes hepatic glycogen stores available as blood sugar for the insulin activity and further metabolism. The presence of this hypothetic additional hormone may account for some of the metabolic discrepancies heretofore encountered in clinical diabetes.

It should be stressed again that no conclusive proof exists that the alpha cell produces the H-G F nor that this is actually a hormone. It is possible, certainly, that this substance produces its effect in these cases because of some abnormality in the hepatic glycogen formation or storage permitting different glycogenolytic agents to act differently. Therefore, the possibility of treating these young labile diabetic patients with the H-G factor in an effort to make their diabetes easier to control, even though larger doses of insulin would then be required, is worth some speculation but such efforts should await further study of other metabolic implications which will be discussed elsewhere.

Conclusions. A small number of patients were treated with the H-G factor obtained as a contaminant of certain insulin preparations. Five of 6 patients with labile diabetes showed a rise in blood sugar greater than was seen in any of the normal subjects or stable diabetic patients.

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Ultraviolet Photography of Paper Chromatograms in the Study of Nucleic Acids.* (17915)

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In the course of studying, by paper chromatography, the enzymatic degradation of DNA and RNA by streptococcal enzymes, a simple method of photographing the paper strips in the ultraviolet was developed, and forms the basis of this report.

Method. Fig. 1 illustrates the mechanical system employed. The ultraviolet light source was a model SL Mineralight† containing a 2537 short wave filter. The paper chromatogram was fastened tautly across an open box or taped to the undersurface of a supported sheet of clear glass (*i.e.*, with the paper facing the light source). Any camera capable of focusing at close distances was found suitable when used with a light yellow filter (*e.g.*, K_2 filter). High contrast film (*e.g.*, Process Pan) was preferable, although overdevelopment of ordinary film produced satisfactory contrast. A distance of 14 inches from light source to paper, and an exposure time of one hour at F 4.5, using a film with a Weston rating of 48, gave consistently satisfactory results. Further distances from the light source gave more even illumination of the paper, but made longer exposure times necessary. With the lighting source described, the method of Markham and Smith(1), which employ contact printing, could be simplified, but was not found to be as convenient in our enzymatic studies.

Results. Fig. 2 illustrates an ultraviolet photograph, by the procedure outlined, of a paper chromatogram of 1% DNA and RNA

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† Ultraviolet Products, Inc., South Pasadena, Calif.

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