sion. Since the concentration in the blood of any substance released from vessel walls must necessarily be a direct function of the ratio of blood vessel surface to blood vessel caliber, one would expect a progressive increase in fibrinolytic activity in serial blood samples collected after venous occlusion.

Aside from a release of fibrinolytic substances from vessel walls, other possible mechanisms must be considered for the production of increased fibrinolytic activity in small vessels during venous occlusion. Destruction of white blood cells could release leucoprotease. Such a mechanism has been suggested by Gotschall(11). The observation that the number of white blood cells is decreased in the postocclusion samples would be compatible with this hypothesis. Another possibility is that contact activation contributes to the increase in blood fibrinolytic activity. Addition of kaolin to plasma *in vitro* enhances greatly plasma fibrinolytic activity, presumably through activation of Hageman factor(12). It is conceivable that venous occlusion could produce alterations in the inner lining of small blood vessels which might cause contact activation of the blood fibrinolytic system. In support of the last hypothesis is the observation that the fibrinolytic response to venous occlusion is smaller in persons with Hageman trait than in normal individuals(13).

Summary. The fibrinolytic activity of serial blood samples, 5 ml each, collected rapidly one after another after 5 or 10 minutes of venous occlusion of the forearm, increases progressively from the first through the third or fourth sample. This observation indicates that small venous tributaries and/or capillaries are a major site for production of increased fibrinolytic activity during venous occlusion.

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Substrate Inhibition of Tetrazolium Salt Reduction in Dark Adapted Retinae.* (29885)

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Enoch(1) reported that the light adapted retina of rat, rabbit, and squirrel monkey reduced more nitroblue tetrazolium chloride (nitroBT) in simple buffered media using succinate as substrate than did the fellow dark adapted retina. The effect was enhanced when the tissue culture medium, TC-199 without phenol red, was used and the effect was reduced if ATP was added to the media. Sickle(2) has reported results which suggest that light stimulation of isolated frog retinae alters metabolism or metabolic pathways.

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Albino rabbits of either sex were dark adapted 2-18 hours. One eye was patched in the dark with a tight fitting metal cup rimmed with a soft rubber gasket before removal to the adapting light source, a 60 W tungsten bulb in a focusing reflector held about 12 inches from the eye. After 10 minutes exposure of the one eye to the adapting light the rabbit was beheaded. In the dark room both eyes were enucleated, and dissected, and the retinas excised, and placed in the incubation mixture. Low intensity, Wratten #29 filtered, light was used during these latter procedures.

The incubations, a modification of Enoch's procedure(1), were carried out in 0.6 ml of media in the 25 mm dia. of a spot plate. The media used had substrate at 10^{-1}-10^{-3} M, nitroBT at 0.75 mg/ml, 0.5 tris-chloride buffer at pH 7.35-7.40, and enough NaCl to make the final media isotonic at 318-320 mOsmol. Incubation time was 10 minutes unless otherwise noted. The temperature during incubation was 21° ± 1°C. The reaction was stopped by addition of 1.0 ml of 5% TCA to the reaction mixture. The retinae were then transferred within ½ minute to 5% TCA. Judgments of the difference, if any, in amount of blue colored formazan deposited in the uninjured parts of the light adapted and dark adapted pair of retinae were made at this point. Colored photographs of the 2 retinae in the same frame were made for the record of most of the experiments. The retinae were then frozen, sectioned with a cryostat microtome, mounted on microscopic slides, and examined under the microscope to determine the localization of the formazan in the retinal layers.

The criterion for a positive difference was agreement of 2 or more observers that one of a given set of retinae was darker blue than the other in comparable areas of the retina with no detectable injuries. Even a small injury increases enormously the amount of formazan deposited. A dissecting microscope was used in doubtful cases. In the event the observers did not agree or could not decide easily, the result was recorded as no difference. The criterion used to decide whether there was a difference with a given substrate was a detectable difference in all of 8 or more trials. The visual pigments remaining in the dark adapted retinae gives them an orange-yellow color which is absent in the light adapted retinae. Although the presence of the orange-yellow color should make the blue colored formazan in those retinae appear more intense than it actually was no attempt was made to correct for this effect in the results reported here.

A difference between the amount of nitroBT formazan stain in the 2 retinae was found in all trials with 5 of the 13 substrates used. Two of the substrates used had apparent differences in more than half of the trials, while 6 of the substrates used showed no difference or differences divided equally between dark adapted or light adapted retinae with the most formazan. In addition light adapted and dark adapted retinae from fellow eyes incubated in media without added substrate showed no difference in the amount of formazan in the 2 retinae. These results and the substrates used are summarized in Table I. Those substrates which gave a difference are listed in the approximate order of the magnitude of the difference with glutamate at 10^{-3} M showing the greatest difference.

In 10 minutes all layers of the rabbit retina will reduce detectable amounts of nitroBT when a concentrated solution of this histochemical reagent is injected *in vivo* into the vitreous near the retina. Excised rabbit retinae incubated 10 minutes in media with minimal nitroBT with or without any substrate listed in Table I, has detectable amounts of blue formazan stain only in 2 layers; the ganglion cell layer, mostly at or near the nuclei of these cells, and in the inner segments mostly at or near the ellipsoids. Practically all of the formazan which was detected in the uninjured areas of these retinae upon microscopic examination was found in the inner segments. Although most of the sections examined with many of the substrates had detectable amounts of nitroBT formazan deposited in the ganglion cell layer, no consistent differences between light and dark adapted retinae were noted. In contrast the amount and distribution of forma-

TABLE I. Results with the Substrates Tested for Effect on NitroBT Reduction in Light Adapted Retinae Compared with Fellow Dark Adapted Retinae.

Substrate	Concentration	Retinae with less formazan	No. difference/ No. of trials
Glutamate	.1–10^{-7}M	Dark Adapted	25/25
Succinate	.01M	" "	9/9
Ethanol	10^{-4}M	" "	11/11
Butanol	10^{-3}M	" "	8/8
Isocitrate	5×10^{-3}M	" "	8/8
Malate	$.01–5 \times 10^{-4}$M	" "	8/10
Lactate with PhMS	10^{-4}M	" "	5/8*
Lactate	10^{-4}M	Neither	0/5
6-phosphogluconate	10^{-3}M	"	4/9
Glucose	10^{-3}M	"	0/5
Glucose-6-phosphate	10^{-3}M	"	0/5
α-hydroxyvalerate	.01M	"	0/5
DL-β-hydroxybutyrate	.01M	"	0/5
DL-β-hydroxybutyrate with PhMS	.01M	"	0/5*
Glutamate with NADH†	.1M	"	0/5
No substrate		"	0/13

PhMS = phenazine methosulfate (10^{-6}M).
* Incubated 5-6 min.
† NADH concentration 10^{-7}M.

zan in the inner segments, particularly with respect to the ellipsoids, was quite remarkable between retinae with differences in the amount of formazan. The most consistent finding was that in all instances in which there was a difference in the amount of formazan stain in a pair of retinae, one light adapted the other dark adapted, the dark adapted retina had less formazan than the light adapted retina.

Light adapted and dark adapted retinae were excised as in the other experiments. Each of these retinae was cut in two. One piece of dark adapted and one piece of light adapted retinae was incubated in complete media using 10^{-3} M glutamate as substrate. The other halves of these retinae were incubated in identical media but without substrate. Incubation time was exactly 10 minutes. Fixation and examination was complete as outlined above. These experiments were repeated with succinate as substrate. Upon examination the dark adapted half retina incubated with substrate was found to have less formazan than the other 3 retinal pieces.

The statement that light adapted retinae reduce more nitroBT than dark adapted retinae implies that some enzyme system is more active in light adapted retinae. Since the experiment reported above suggests that a non-specific, substrate independent, reduction of nitroBT is inhibited by the presence of glutamate or succinate, but only in the dark adapted retinae, it is more precise to report the results as formulated in this paper, namely as substrate inhibition of nitroBT reduction.

The inhibition of nitroBT reduction by glutamate was investigated over the concentration range from 0.1 M to 10^{-8} M. No inhibition was found with the 10^{-8} M glutamate, but 10^{-7} M and all higher concentrations were definitely inhibitory. Glutamate concentrations above 10^{-3} M appear to inhibit nitroBT reduction somewhat even in the light adapted retinae. This effect is similar to the effect of ATP reported by Enoch with succinate as a substrate and also to the effect of 2.5 minutes preincubation with 10^{-7} M NADH using glutamate as a substrate since with the NADH present it took a longer incubation time (15 minutes) to give a visually detectable amount of formazan.

Addition of 10^{-6} M phenazine methosulfate to the media shortens the time necessary to give a visually detectable amount of formazan from 10 minutes to 6 minutes. Phenazine methosulfate was required in the media when lactate was used as substrate in order to be able to detect a difference between the light and dark adapted retinae.

When the temperature of the incubation was increased to 30°C the difference in

amount of formazan deposited in the light and dark adapted retinae with glutamate as substrate was less and the total amount of formazan stain in the retinae appeared to be greater. An increase in time of incubation from 10 to 15 minutes with the substrates tried, succinate and glutamate, increased the amount of formazan so much that any difference between the light and dark adapted retinae could not be detected visually. Additional nitroBT in the media has the same effect. These results suggest that any difference observed is due to a constant difference in amount of formazan deposited and that some co-factor or electron transport carrier is altered. An alternative hypothesis is that the light intensities used damage some enzyme system in the extra mitochondria space which inhibits certain electron transport reactions within the mitochondria where nitroBT reduction is thought to take place (3). The present data do not permit one to distinguish between these alternatives. However, these data do suggest that light of the intensity used does not alter appreciably the permeability either of the cells or the mitochondria because some substrates used did not show the effect. Since all of the substances which seem to inhibit the reduction of nitroBT in the dark adapted retinae are substrates for enzymes which do or can use NAD as a coenzyme and since NADH preincubation also inhibits nitroBT reduction in both dark and light adapted retinae, one is tempted to speculate that NADH production by any enzyme system present will produce the effect. The data in Table I do not fully support such a conclusion since substrates for some NAD requiring enzymes show no effect.

The effect reported here appears to be specific for the retina because it could not be demonstrated in excised iris, or cerebral cortex tissue of the rabbit.

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Effect of Hypoinsulinism on Intracellular Transport of Glucose in the Small Intestine. (29886)

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One of the mechanisms of action of insulin suggested is that it facilitates the entry of glucose inside the cells(1-4). Insulin has been found to increase the transfer of pentose (5), amino acids(6) and potassium(7) from the extracellular fluid to the intracellular fluid. Thus insulin is necessary for intracellular transport of glucose, pentose, amino acids and potassium. The intestinal absorption of glucose involves transference of the sugar from the mucosal to the serosal side of the intestine. Absorption of glucose from the intestinal lumen, however, increased in insulin deficiency produced either by injection of alloxan in rats(8,9) or by development of scurvy in guinea pigs(10). It has been reported that scorbutic guinea pigs suffer from hypoinsulinism(11).

Materials and methods. Albino rats and guinea pigs weighing between 250 g and 300 g were used. Rats were made diabetic by intraperitoneal injection of alloxan, 200 mg/kg body weight. Scurvy was produced by feeding guinea pigs a scorbutogenic diet(12) for 4 weeks.

After a fasting period of 24 hours, normal guinea pigs, scorbutic guinea pigs, normal rats and alloxan diabetic rats were killed by stunning followed by decapitation. The small intestine was removed, flushed with ice-cold Ringer fluid, everted, cut into pieces of 5 cm in length starting from the duodenal end and