broncho-constrictor agent from the smoke. As the cigarette became shorter (5 cm), the retained (and possibly concentrated) broncho-constrictor agent may be volatilized by heat of the burning tip of the cigarette so that it is again carried along in the smoke. As the cigarette becomes still shorter (3 ml) an insufficient length of unburned tobacco may remain to act as an efficient filter.

Summary and conclusions. Experiments on intact guinea pigs indicate that cigarette smoke contains a broncho-constrictor agent. The intensity of the broncho-constrictor response is different with different lengths of cigarettes. Evidence is presented which indicates that the active agent in the smoke is not nicotine. Experiments using 2 brands of filter-tipped cigarettes indicated that the filter-tip did not influence the response of the lung to the cigarette smoke. The single brand of "denicotinized" cigarettes showed significantly less total broncho-constrictor effect than did the conventional cigarettes.

The author wishes to thank Mr. Willard Bloodworth for capable technical assistance.

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Hypoglycemic Action of Orinase.* Effect on Output of Glucose by Liver. (22471)

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Bondy(1) demonstrated in humans that during postabsorption the output of glucose from the liver into the hepatic vein is in continuous state of flux, its level varying grossly and unpredictably from moment to moment. Bondy employed angio-catheterization of the hepatic vein, a method precluding sufficiently free flow of blood for precise short-interval timing required for methodology presently under consideration. We have explored these aperiodic fluctuations in normal dogs by exposing hepatic veins and directly needling the same for blood-glucose determinations at 1-minute and at 15-second intervals(2). When these readings are plotted, they assume varying configurations by their total irregularity both in rhythm and in magnitude. When similar readings are made on blood from the femoral artery, the same irregular glucose fluctuations are observed peripherally(2). Circulation time from the hepatic vein to the femoral artery was performed with fluorescine under a Wood lamp. Using this determination, one can readily identify the peripheral femoral arterial fluctuations with those emanating from liver. Irregular configuration of the hepatic undulations makes possible this peripheral identification (Fig. 1a). The original hepatic fluctuations are grossly reflected at lower over-all glucose levels throughout the main arterial tree of all 4 extremities in wavelengths of 2 to 7 minutes. When these relatively long "undulations" at both points are broken down into their structural components by taking glucose readings at 15-second intervals, one notes a markedly exaggerated variation from reading to reading, which is registered as a series of sharp flings, up and down (Fig. 1b). These may aptly be designated as "oscillations," each complete "oscillation" taking approximately 30 seconds. The

* Orinase-Upjohn (1 butyl-3p-tolylsulfonylurea) supplied by Upjohn Co. through courtesy of Dr. C. J. O'Donovan. Glucagon supplied by Eli Lilly Co. through courtesy of Drs. F. B. Peck and W. R. Kirtley. The authors are also indebted to Agnes Dann for technical assistance in 2000 glucose determinations.

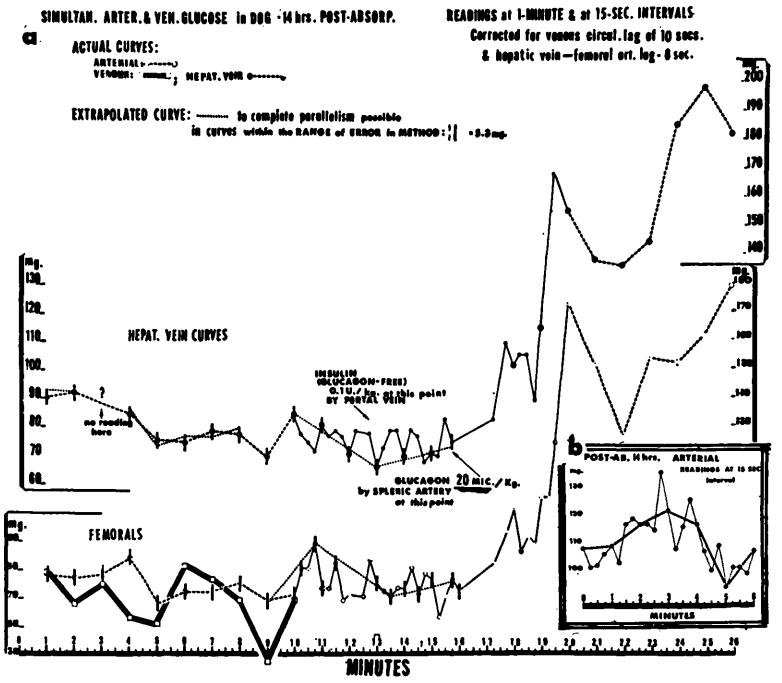

FIG. 1. a. Note parallelism of "undulations" within range of error. Component "oscillations" lose identifying configurational characteristics by passage through cardio-pulmonary circuit. b. Note wide sharp flings of "oscillations," readings at 15-sec., components of "undulations."

consistent presence of these sharp "oscillations" in hepatic venous blood throughout the period of postabsorption would suggest that during all intervals between absorption of nutrients, glucose is constantly discharged from the liver(2). At these times, however, the release occurs in sharp brief aperiodic spurts. The writers have found evidence to suggest that these aperiodic spurts of glucose from the liver are probably of glucagon origin and that they perform the physiologic function of triggering insulin into production and action during postabsorption much as does ingested glucose during the stage of absorption(3). Such fluctuations in the peripheral arteries may safely be presumed to reflect the degree and character of output of glucose by the liver into the hepatic vein. A diminished output of glucose by the liver would be anticipated to register itself during postabsorption not only as a lowered over-all arterial glucose level but also in a diminution or even a complete obliteration of the more gross undulations normally exhibited in the peripheral arteries, just as occurs after hepatectomy(2).

These principles have been applied to a study of the character of glucose output by the liver in dogs which have been subjected to intensive medication with the aryl-sulfonylurea, Orinase-Upjohn (1 butyl-3p-tolylsulfonylurea).

Procedure. For a control period of 10 days, normal dogs were fed a liberal mixed diet which included 1 to 2 lbs of beef liver daily. They were then anaesthetized (14 hours postabsorptively) with Sod.-pento-barbital by vein and heavily ergotaminized with dihydroergotamine tartrate (0.5 mg/kilo by vein). Control glucose† curves were established simultaneously on arterial and venous blood from a single extremity, the venous circulatory lag having been predetermined by an arterio-venous fluorescine circulation time

† Somogyi-Nelson(4) macro method in duplicate. Mercury-standardized pipettes. Standard error in methodology 5.3 mg/100 ml. Hematocrits followed throughout all procedures.

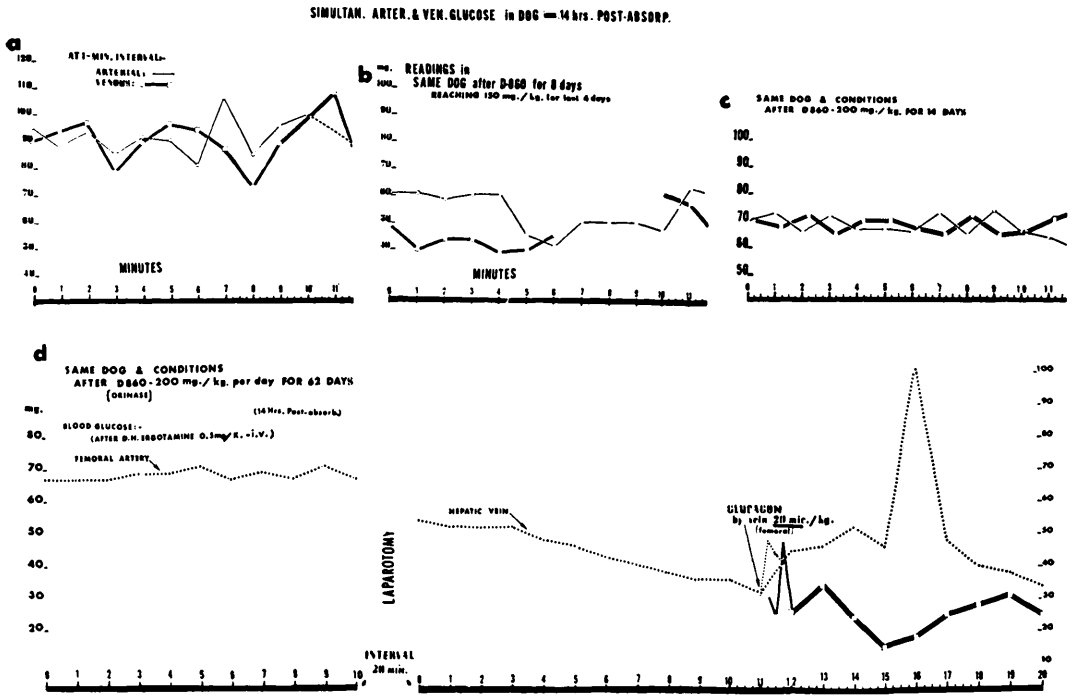

FIG. 2. Fine lines represent arterial curves. Disregard venous curves (heavy) which are irrelevant to present consideration. Glucagon I.V. at the end of curve 2c (not recorded) reestablishes arterial curve 2a in exaggerated form. Note return of hepatic undulations in 2d by glucagon I.V.

(Fig. 2a). Venous readings were ultimately found to be irrelevant to the problem at hand and although recorded are not considered. 1. One animal received Orinase orally for 8 days in progressively increasing dosage. Original blood sampling procedure was then repeated (Fig. 2b). 2. For the next 14 days the same dog was given the drug (200 mg/kilo/day), whereupon the initial sampling procedure was repeated (Fig. 2c). On completion of these readings, glucagon (20 μg/kilo) was given by vein (not recorded in Fig. 2c). 3. Thereafter, the same dosage of drug was continued for 48 days (total period of intensive drug administration being 70 days). After peripheral arterial blood sampling, the animal was laparotomized for direct sampling from the hepatic vein (Fig. 2d). The dog was then sacrificed, specimens from pancreas, adrenals, thyroid, liver and kidneys having been removed previously from the living animal for histologic examination. 4. A second dog was studied in similar manner to determine the acute effects of Orinase, the sodium salt of the drug being administered by vein in dosage of 100 mg/kilo (in 2 minutes). One hour after administration, femoral arterial blood was sampled at 1-minute intervals (Fig. 3a). Immediately thereafter, the animal received by vein in zero time 100 mg of the drug together with glucagon (10 μg/kilo), the mixture having been allowed to stand at 37°C for 1 hour. The 1-minute blood sampling was resumed. A second dose of glucagon (10 μg/kilo) was then administered by vein and the blood sampling continued. 5. For the next 14 days the drug was withheld. The animal was then again heavily ergotaminized and laparotomized (Fig. 3b) to obtain hepatic venous blood samples. Five ml of blood was withdrawn from the hepatic vein, evenly and steadily with stop-watch precision (1 ml/min.) and totally quantitated for its glucose content (Fig. 3b). The drug (150 mg/kilo) was then administered by vein over 2 minutes time. Ten minutes later, a second 5-minute glucose output rate by the liver (mg/100 ml of blood) was determined. Shortly

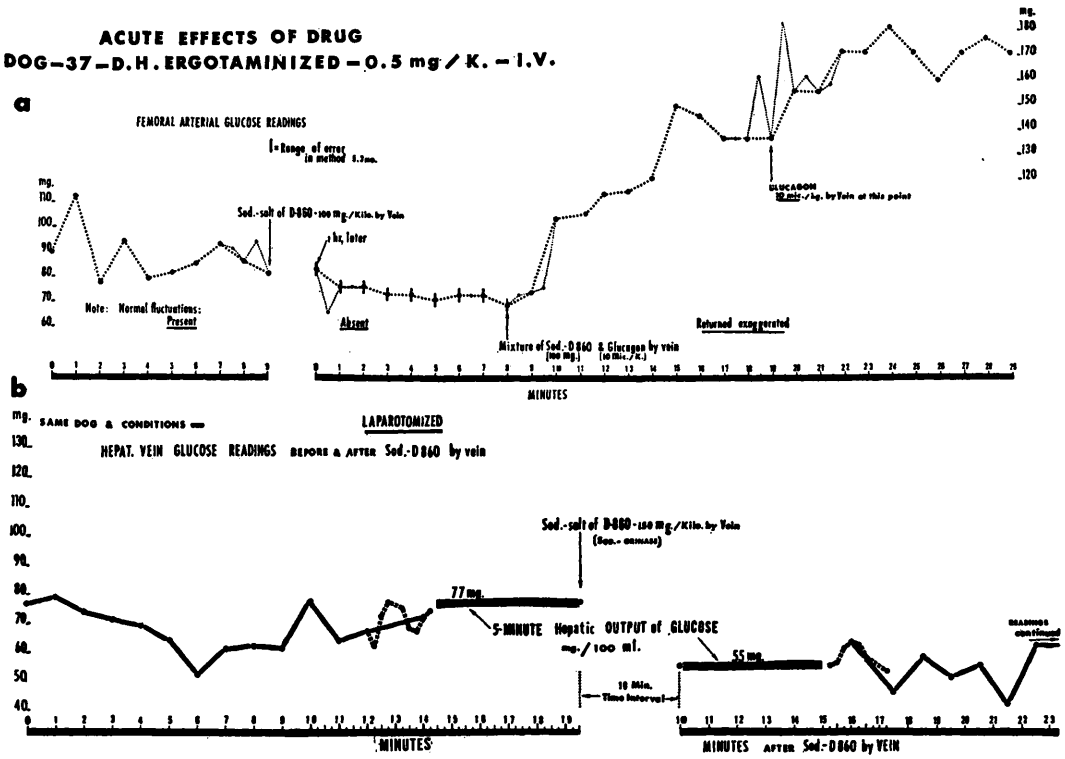

FIG. 3.

thereafter glucagon (5 μg/kilo) was given by vein in zero time (Fig. 4) and 10 minutes later a second dose of glucagon (15 μg/kilo) was administered. Shortly thereafter the animal was sacrificed for tissue examination as in the first dog.

Findings. 1. Fig. 2 exhibits a fall in mean glucose level and a progressive loss of normal peripheral arterial undulations under the influence of the sulfonylurea. After 14 days of oral exposure to the drug, arterial undulations outside the range of error have completely disappeared (Fig. 2c). These findings suggest that during postabsorption the character of delivery of glucose from the liver as well as its rate of output by that organ have changed as a result of the drug. The dose of glucagon (20 μg/kilo) which was given by vein shortly after completion of the readings in Fig. 2c resulted in a prompt re-establishment of the pre-drug arterial undulations but in markedly exaggerated form (not included in this Fig.). Accordingly, even after 14 days of intensive exposure to the drug, glycogen is still abundantly available in the liver for this exaggerated phosphorylative glycogenolysis.

2. After 70 days exposure in large dosage (200 mg/kilo, over 14 times the average daily therapeutic dose in humans), normal hepatic venous and peripheral arterial glucose fluctuations have been completely obliterated (Fig. 2d). They are, however, still capable of immediate re-establishment by a dose of glucagon by vein. Presumably the functional integrity of the phosphorylase enzyme systems I and II has in no wise been compromised (or, at least, irreversibly so) by the drug. The animal lost weight despite heavy ingestion of food. For the last 5 days before sacrificing, he showed considerable ataxia on walking and seizing food. The pancreas (including alpha-beta cell differential count), the adrenals, thyroid, liver and kidneys were normal to histological examination.

3. Orinase given by vein acts very promptly to produce changes in glucose output by the liver. Within one hour after exposure (Fig.

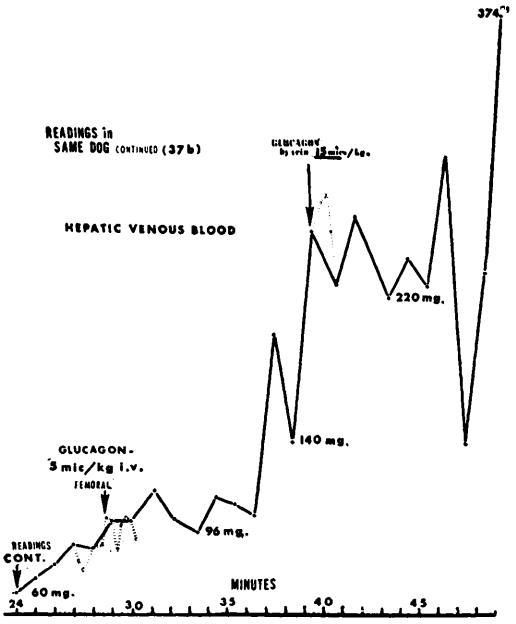

FIG. 4. Same dog as in Fig. 3. Note escape from hypoglycemia between 24th and 29th min., greatly intensified by a small dose of glucagon I.V.

3a), there developed both a fall in blood-glucose level and complete loss of normal pre-drug peripheral arterial undulations. When a mixture of the drug with glucagon was given by vein, there occurred no recognizable inhibitory effect by the drug on normal glycogenolytic effect of administered glucagon. Within 10 minutes time after a large dose of the drug by vein (Fig. 3b), there occurred a sharp decline in quantitative output of glucose by the liver (33%). The normal hepatic glucose undulations have been reduced somewhat in amplitude but have not been obliterated, their complete loss presumably taking place at some time between the 10- and 60-minute points. At the 10-minute point, the intrinsic glucagon mechanism of the ergotaminized animal is still functioning as is indicated by a persistence of the glucose fluctuations in hepatic venous blood and by the animal's spontaneous rebound or escape from hypoglycemia between 24th and 29th minute (Fig. 4). This escape is greatly enhanced by a small dose of glucagon (5 μg/kilo I.V.). Existent fluctuations are powerfully accentuated by this small dose of the hormone but extremely intensified (over 400%) by a second dose of 15 μg/kilo.

Discussion. The hypoglycemic effects of Orinase are evidently mediated, if not entirely, at least in large part, through its capacity to reduce output of glucose by the liver. There is quantitative evidence of reduction of such output within 10 minutes after intensive intravenous exposure to the drug. Within one hour after such administration, the normal minute-to-minute postabsorptive glucose fluctuations have completely disappeared and have been replaced by a flat glucose curve.

That the phosphorylase enzyme systems I and II are functionally intact after 70 days of intensive exposure to the drug is indicated by the fact that even a minute dose of glucagon is still capable of activating immediately and completely these systems to a state highly exaggerated over their pre-drug activity. The peripheral glucose fluctuations normally found in arterial blood are grossly wiped out by the drug. Since these directly reflect the character of phosphorylative glycogenolysis taking place in the liver (and expressed by output of glucose into the hepatic vein), the block in the chain of events must be proximal to the phosphorylase systems I and II. Any possible adrenal medullary effect on these enzyme systems had already been abolished by heavy ergotaminization.

A suppression in the production of glucagon at source may very well account for the observed inhibition of glycogenolysis. The prompt return of glycogenolysis on administration of a minute dose of glucagon suggests this concept. Our findings do not rule out the remote possibility that Orinase may obstruct the function of other enzyme systems which may also be proximal to the phosphorylase systems and essential links in the glycolytic effect of glucagon. No one has, however, identified any such system, nor would the effects we have observed seem to fit the functional description of Mirsky's insulinase-insulinase-inhibitor systems(5,6).

The tendency of the normal animal and of the clinical diabetic to become more sensitive to glucagon after exposure to the drug fits better with a reasoning that the drug has

caused an absolute deficiency of glucagon itself, a suppression of the hormone at source, wherever this may be. An absolute deficiency of a hormone is frequently reflected by an increased sensitivity to that hormone(7). The exaggerated physiologic response to a small dose of glucagon fits in with an absolute deficit of this hormone produced by the drug. We have found by contrast that exposure to the drug causes no significant change in the body's responsiveness to insulin. This is also in contrast with the improvement in insulin sensitivity observed in obese diabetics after adequate dietary treatment alone(7).

The speed with which the glucose lowering effect of the drug is established (10 minutes) and reversed suggests reversible interplay of enzyme systems. The anatomical integrity of the alpha cells after 70 days of intensive exposure does not support the original claim of the German workers that the drug functions by destruction of the alpha cells.

Conclusions. 1. A simple method for gauging changes in the output of glucose by the liver has been described. Throughout postabsorption, there are present in hepatic venous blood aperiodic fluctuations in glucose which are reflected throughout the major peripheral arterial tree. Under the influence of the drug, these fluctuations at both sites promptly disappear. The quantitated changing rate of output of glucose by the liver after Orinase (33% fall in 10 minutes) is drastic. A small dose of glucagon by vein immediately reverses this trend with a return in exaggerated form of the normal postabsorptive fluctuations. As a corollary one may infer that long use of the drug does not impair liver glycogen deposition. 2. It is suggested that the site of action of Orinase is proximal to the phosphorylase enzyme systems and takes the form of a suppression of glucagon production at source with a resulting reduction of glucose output by the liver. After 70 days of intensive exposure to the drug in the normal dog, acute signs and symptoms of toxicity are present but no histopathologic changes in the liver, kidneys, thyroid, adrenals or pancreas (including alpha-beta cell ratios). 3. No evidence was found to suggest that the drug works by destruction of the alpha cells of the pancreas.

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Pulmonary Vascular Changes in the Mouse During Anaphylactic Shock.* (22472)

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Discussions of anaphylaxis generally emphasize the differences in reactions in various species of animals. However, Burrage and Irwin, by direct microscopic observation of the living pulmonary circulation, have shown a marked similarity in changes occurring during anaphylaxis in the rabbit and the guinea pig(1). They noted pulmonary constriction, clumping of red cells and embolization in both species. This article will describe pulmonary vascular changes in the mouse during anaphylactic shock. The methods employed in observing pulmonary circulation were essentially the same as those of Burrage and Irwin(1).

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