ported that adaptation of poliovirus type I from HeLa cells to the egg was successful only through inoculation of the virus with HeLa cells but did not succeed with cell-free infected culture fluid. At present it is not certain that our new adapted strain can be cultured serially in established S180 cells. Among the Columbia SK virus group, propagation of Mengo and EMC viruses in mouse ascites cells was reported by Koprowska and Koprowski(8), Flanagan and Colter(9) and Hoskins and Sanders(10). These viruses grew easily from the original brain viruses without necessity of adaptation, but this was not possible with the Columbia SK virus. They did not describe HA in their systems. We have found HA a useful addition to CPE and infectivity in studying the effects of antiviral drugs because it tends to be more specific than CPE and more rapid and quantitative than infectivity titration.

*Summary.* Adaptation of Columbia SK virus from Ehrlich ascites cells to Sarcoma 180 ascites cells *in vivo*, and the serial passage *in vitro* are described. The adaptation was successful only through the use of mixtures of both cells. The new adapted virus propagates efficiently in Sarcoma 180 ascites cells *in vitro* with CPE and production of HA. This virus-host system has been used for screening tests of antiviral drugs.

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## Relation of Potassium and Glucose Release from the Liver in the Unanesthetized Dog.* (26905)

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An interrelationship between potassium and glucose movement has long been noted. Fenn(1) has described the deposition of glycogen in a matrix of potassium and phosphate ions, and has shown that glycogen can not be deposited unless accompanied by appropriate amounts of potassium and water. D'Silva(2) noted transitory increases in plasma potassium with glycogenolysis induced by epinephrine in the intact animal and in the perfused cat liver(3). This has been confirmed(4,5,6,7). However, it has also been demonstrated that epinephrine produces a small increase in blood potassium when the liver is out of the circulation(8,9). Moreover, epinephrine also causes the perfused dog lungs to release potassium(10).

Since glycogen deposition is accompanied by potassium accumulation in the liver, D'Silva(11) suggested that glycogenolysis must result in potassium release from the liver.

In the present study concentrations of potassium and glucose of plasma entering and leaving liver were measured in unanesthetized dogs before and after glucagon and epinephrine administration. Concentration differences across the organ were multiplied by the simultaneously measured hepatic plasma flow rate to give an approximation of net

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rate of hepatic potassium and glucose uptake or output.

*Methods.* Sixteen experiments were carried out in mongrel dogs whose hepatic vessels were catheterized with large plastic tubing by a previously described technic(12), 2 to 7 days prior to the experiment. All dogs were studied in an unanesthetized state without sedation after an overnight fast. Arterial, portal and hepatic venous blood was withdrawn by a constant withdrawal pump so that 4 to 6 contemporaneous, time-integrated control samples were obtained over 5- to 10-minute intervals. After rapid intravenous administration of glucagon 0.02 mg/kg or epinephrine 1 μg/kg into a systemic vein, samples were obtained at 10-second intervals for 2 minutes and at less frequent intervals thereafter.

Hepatic plasma flow was measured by a modified bromsulphalein method(13). Plasma potassium was determined by an internal standard flame photometer and plasma glucose by the Somogyi-Nelson method(14). Hepatic output of potassium or glucose was calculated as product of concentration differences (hepatic venous minus portal venous concentrations) and hepatic plasma flow rate.

*Results.* A. *Effect of glucagon.* 1. *Hepatic venous, arterial and portal venous potassium concentration.* The hepatic potassium response to glucagon occurs rapidly. At 20 to 30 seconds after glucagon injection elevation of hepatic venous plasma potassium began. This elevation usually became maximal between 1 and 2 minutes after injection and returned toward control values within 5 to 10 minutes. At its maximum the mean elevation in hepatic venous plasma potassium was 1.2 meq/l above control (Fig. 1).

Lesser increases of arterial and portal venous plasma potassium occurred immediately after hepatic venous elevations.

2. *Hepatic-portal venous plasma potassium concentration difference.* The hepatic-portal venous concentration difference increased from a mean control value of 0.1 meq/l to a mean maximum of 0.7 meq/l after glucagon administration (Fig. 1).

3. *Hepatic potassium output.* Mean control hepatic potassium output was .08 meq/min. There was a markedly increased rate of potassium output from the liver after glucagon administration. Between 1 and 1.7 meq of potassium were released by the liver in the first 2 minutes after glucagon. This potassium release roughly paralleled the increased potassium concentration difference across the organ (Fig. 1).

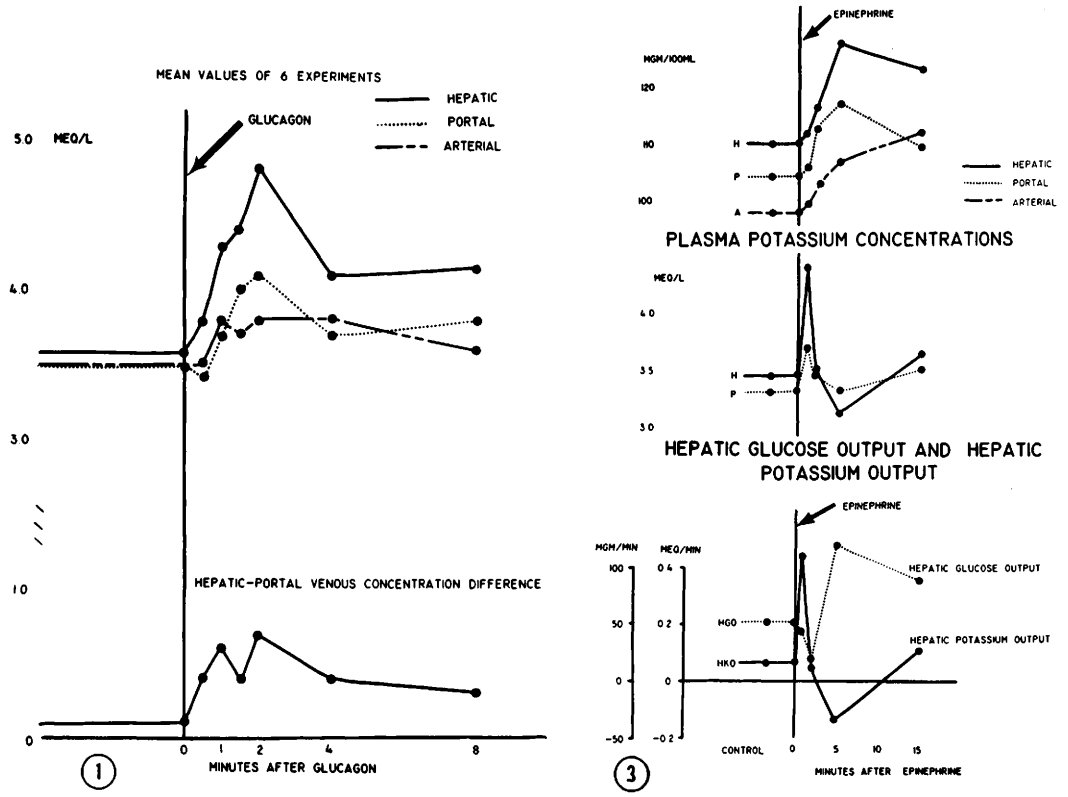
4. *Late potassium movements.* During the ensuing hour there was a gradual fall of potassium in arterial, hepatic venous and portal venous plasma. Hepatic-portal venous concentration difference decreased. The initial output of potassium by the liver gradually fell to become negative; *i.e.*, hepatic potassium uptake.

B. *Effect of epinephrine.* 1. *Hepatic venous, arterial and portal venous plasma concentrations.* Hepatic venous plasma potassium concentrations increased markedly and rapidly after epinephrine administration. The pattern was similar to that following injection of glucagon except that the epinephrine response was shorter in duration. In most instances, return to normal or near normal levels occurred within 120 seconds. Fig. 2 illustrates a representative experiment.

2. *Hepatic-portal venous plasma concentration difference.* The potassium concentration difference across the liver was markedly increased after epinephrine injection. Following this increased positive gradient, a pronounced negative gradient was consistently observed.

3. *Hepatic potassium output.* Rate of hepatic potassium output is represented as a function of time after intravenous epinephrine injection (Fig. 3). Except for being more rapid, the pattern of potassium output from the liver after epinephrine was similar to that following glucagon administration. A total of .4 to .8 meq potassium was released by the liver after administration of epinephrine, 1 μg/kg. Hepatic uptake of a similar amount of potassium followed. An increased hepatic plasma flow began during or immediately after onset of hepatic potassium output.

4. *Hepatic glucose output.* Concentration of glucose in arterial, portal and hepatic ve-



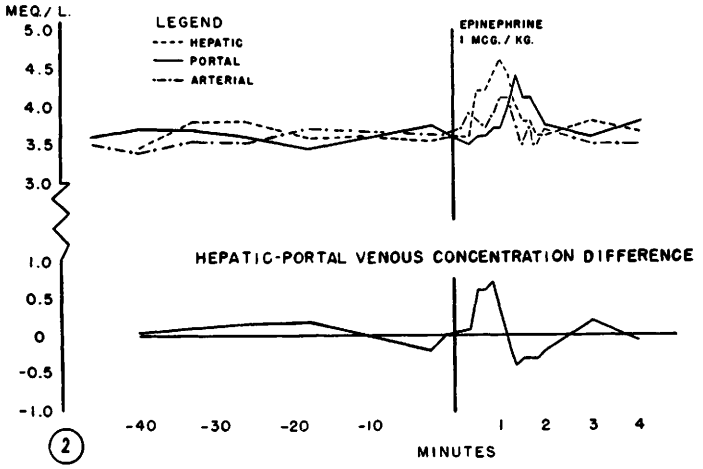




FIG. 1. Arterial, portal and hepatic venous plasma potassium concentrations and hepatic-portal venous concentration differences of a series of 6 dogs before and after glucagon, .02 mg/kg. There is a marked and rapid rise in hepatic venous potassium levels and in hepatic-portal venous concentration differences appearing within 30 sec. and reaching a maximum 2 min. after inj.

FIG. 2. Arterial, portal and hepatic venous plasma potassium concentrations and hepatic-portal venous concentration differences before and after epinephrine, 1 $\mu$g/kg. The pattern of potassium flux is similar but more rapid than that occurring after glucagon administration.

FIG. 3. Concentrations of plasma potassium and glucose, and net flux or output, in a representative experiment in which epinephrine was administered. There is a marked output of potassium prior to an increase in hepatic glucose output.

nous plasma as well as rate of hepatic glucose output are compared with concomitantly measured potassium values in a representative experiment (Fig. 3). Release of potassium by liver, preceded the earliest increase in glucose output.

*Discussion.* Measurement of rates of net movement of potassium and glucose as a function of time after epinephrine or glucagon injection indicates the sequence of events occurring with hormone-induced glycogenolysis. Potassium is first released by the liver. An increased hepatic glucose output follows 3 to 6 minutes after epinephrine injection. We(15) have previously reported that maximum hepatic glucose output occurred 10 to 20 minutes after glucagon administration.

Of further interest is the observation reported from this laboratory that hepatic blood flow increased after glucagon(16) and epinephrine(17) injection. Hepatic blood flow increases occurred during or immediately after the increased hepatic potassium output following administration of epinephrine. Hepatic blood flow response to glucagon, however, appeared to parallel the increased rate of hepatic glucose output.

The findings of this study refocus attention on the mechanism of glucose release, and strongly suggest that potassium does not merely passively accompany glucose, water and phosphate ion in mass departure from the intracellular phase. Rather, the potassium release is an action well antecedent to that of glucose release, and therefore would appear to be involved in reactions earlier in the glycogenolytic sequence than the actual release of glucose.

Sutherland and coworkers(18) have demonstrated some of the mechanisms whereby glucagon and epinephrine affect transformation of inactive phosphorylase to active phosphorylase. Since muscle phosphorylase has been shown to be potassium dependent, it seems possible that an ionic movement such as that observed in this study may be involved in the primary action of these hormones upon hepatic glycogenolytic enzyme systems.

*Summary.* The time course of hepatic metabolic and hemodynamic events after hormone-induced glycogenolysis is described in the unanesthetized dog. Increased concentration of hepatic venous plasma potassium and rate of hepatic potassium output occurred after intravenous administration of glucagon, .02 mg/kg. These effects were noted within 20 to 30 seconds after injection and became maximal within 2 minutes. Increased rates of hepatic blood flow and hepatic glucose output occurred 10 to 20 minutes after glucagon administration. A similar but more rapid pattern was observed after intravenous administration of epinephrine, 1 μg/kg. Maximum hepatic venous plasma potassium and hepatic potassium output was reached at about 1 minute after injection. Increased hepatic blood flow occurred during or shortly after increased hepatic venous potassium concentration. Increased rate of hepatic glucose output did not begin until after the potassium efflux from the liver. Hepatic glucose output reached a maximum 3 to 6 minutes after epinephrine injection. The possible significance of potassium efflux in the sequence of glycogenolytic reactions is discussed.

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## The Red Blood Cell: An Essential Component of the Toxicity of Strangulation Intestinal Obstruction. (26906)

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It has been demonstrated by several investigators that the peritoneal exudate from dogs which have a strangulated loop of intestine is lethal when given to normal animals(1,2). Such fluid is not always fatal and we have attempted to find out why.

Our data from many dogs and rats revealed that 2 elements were constantly present in any strangulation fluid that was subsequently lethal to normal recipients: an *E. coli* level of at least $10^6$ bacteria/ml and a hemogobin level of at least 3 g %. It did not appear that the presence of other bacteria regardless of concentration made any detectable difference. It did appear, however, that if the *E. coli* level fell below that stated, or if the hemoglobin level fell below 3 g % the fluid began to lose its lethal effect. The following experiments were designed to test this hypothesis.

*Methods.* If the above hypothesis is correct it should be possible to manufacture this lethal fluid in the laboratory. The following groups of experiments were done and the results checked by intraperitoneal injection of the fluid into the rat in a dose of 5 ml/kg. This is the same quantity of fluid used in our earlier experiments. All studies were carried out in male rats of the Sprague-Dawley strain and weighing 200-250 g.

I. The first group of 45 rats were given 5 ml/kg of a 24-hour broth culture of *E. coli*. The inoculum was adjusted to contain $1 \times 10^8$ organisms/ml.

II. The second group of 15 rats was given whole blood intraperitoneally 5 ml/kg.

III. The third group of 29 rats was given an intraperitoneal injection of *E. coli* plus whole blood. *E. coli* were grown in nutrient broth for 24 hours in presence of added whole blood so that hemoglobin concentration was at least 4 g %.

IV. The fourth group of 40 rats was given an I.P. injection of $10^8$ *E. coli* grown in nutrient broth to which had been added rat plasma. The plasma was that amount present in the blood with a hemoglobin concentration of 4 g %. The amount of whole blood necessary to achieve this level was determined, then centrifuged and the resultant supernate added to the broth culture.

V. The fifth group of 174 rats received an I.P. injection of *E. coli* ($10^8$) in nutrient broth to which 4 g % of hemoglobin had been added in the form of washed red cells (red blood cells were separated by centrifugation and washed 3 times with saline solution).

VI. The final group of 10 rats were given an I.P. injection identical to group V except that *Clostridia welchii* replaced *E. coli*.

The number of bacteria injected was estimated in a Coleman Jr. spectrophotometer at 660 m$\mu$. The actual number of viable bacteria injected was determined by subsequent dilution and plating of an aliquot of the injected culture. Quantitative counts were performed in a Quebec colony counter at 24 hours.

Hemoglobin level was determined using the cyanmethemoglobin technique read in a Klett colorimeter using a 420 filter.

*Results.* The results are summarized in Table I. When *E. coli* or the hemoglobin solutions were given independently of each other there was no lethal effect. If the 2