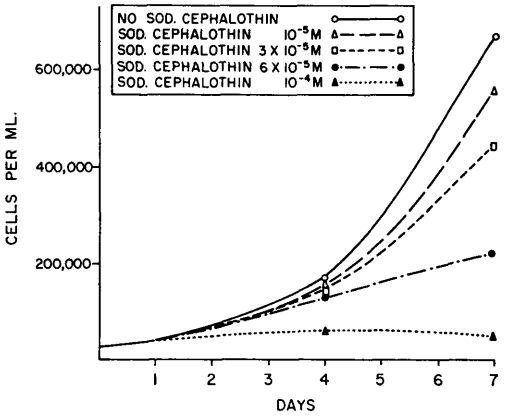


FIG. 1. Effect of sodium cephalothin on growth of McCoy cells in culture.

microbial drugs might be more injurious to mammalian cells than narrow-spectrum antimicrobial agents. The biochemical lesion produced in cultured mammalian cells by cephalothin is unknown though the toxic effects are produced by concentrations suitable for bacterial inhibition.

The demonstration of marked toxicity of cephalothin for McCoy cells in the present study and the findings of significant toxicity of the drug for human amnion and mouse embryo cell cultures(4,5) indicate a need for further studies on the potential toxicity of cephalothin. Caution would seem indicated in the use of this agent in patients with impaired renal and hepatic function in whom very high blood levels of the drug might accrue, in pregnant women because of its unknown teratogenic possibilities, in patients where tissue repair is important, etc.

*Summary.* Cephalothin, in concentrations present in patients on treatment with the drug, produced a marked inhibition of growth of the McCoy strain of cultured human cells and morphologic evidence of toxicity.

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## Disappearance of DL-Lactate-2-$C^{14}$ from Blood in Normal and Diabetic Rats.* (32325)

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Recent recognition of the syndrome of lactic acidosis, which may develop in diabetes, has engendered interest in lactic acid and pyruvic acid metabolism in this disease. The possibility that there is an abnormal metabolism of lactate and pyruvate in diabetes was demonstrated by *in vitro* experiments with diaphragm(1) and cardiac muscle(2). However, the question whether lactate can be metabolized in a normal manner by diabetic animals or humans has not yet been extensively studied.

Shreeve *et al*(3) and Anderson *et al*(4) reported that the blood lactate was slightly elevated in diabetic subjects. The rise in this product of glycolysis represents either increased production or diminished disposal, or a combination of these two factors, and such imbalance might be a contributory cause

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to the lactic acidosis occasionally seen in diabetic subjects. Shreeve *et al*(3) reported mild lactic acidemia which was associated with a decrease in elimination of DL-lactate-2-$C^{14}$ from the blood of severely diabetic patients. Recently, Steenrod *et al*(5) studied the metabolism of lactate in alloxan diabetic acidosis using L-lactate-1-$C^{14}$, DL-lactate-1-$C^{14}$ and DL-lactate-2-$C^{14}$ and found that in diabetic animals, a greater portion of the carbon atoms of lactate was incorporated into urine glucose and less converted to $CO_2$. They concluded that lactate was metabolized at essentially the same rate in both normal and diabetic animals, although in their report, the disappearance of $C^{14}$-lactate over time and the appearance of $C^{14}$ in blood glucose were not measured.

This paper reports the findings on the disappearance of $C^{14}$-labelled lactate from the blood of normal and alloxan diabetic rats, and the concomitant appearance of $C^{14}$ in expired $CO_2$ and blood glucose.

*Methods.* Male Wistar rats weighing about 200 g were used. Diabetes was produced by a single injection of 40 mg/kg of alloxan intravenously, and the rats were used 7 days after injection. Diabetic rats had blood glucose ranging between 300 and 500 mg/100 ml in the fed state 6 days after alloxan injection. After 16 to 18 hours fasting, rats were given pentobarbital anesthesia in order to avoid possible changes in the blood lactate by excitement or motor activity.

The experiments were carried out as follows: 5 $\mu$C/100 g of DL-lactate-2-$C^{14}$ was given intravenously within a few seconds. Immediately after that, the rat was placed in a metabolic cage. In one group of rats, blood samples were taken from the tail vein at 3, 15, 30, and 45 minutes after injection of DL-lactate-2-$C^{14}$ for measurement of the specific activity in the blood lactate. The other group of rats was kept in a metabolic cage under fluothane (2-bromo-2-chloro-1;1;1;-trikuoroethane) anesthesia for 4 hours. Room air was drawn through a carbon dioxide absorbent and then through the cage by means of a vacuum pump. Fluothane anesthesia was induced by a part of the air bubbling through the fluothane solution, adjusting the concentration of anesthetic at about 0.5%. Blood samples were taken every 2 hours from the tail vein. The total $CO_2$ trapped in 1 N NaOH was determined every hour for 4 hours. $BaCl_2$-$NH_4Cl$ reagent was added to a known portion of each collected sample to precipitate $BaCO_3$. Blood glucose was determined by the method of Somogyi-Nelson(6). Glucosazone was prepared from the blood glucose and assayed for $C^{14}$(7). Lactate in the blood was determined by the method of Barker-Summerson (8). Blood lactate after the separation by Dowex 1 $\times$ 1, was oxidized to acetaldehyde and the acetaldehyde was precipitated as its dimedone derivative(9). Radioactivity was measured using a Nuclear Chicago gas flow counter.

*Results.* Fig. 1 shows the time curve of the concentration of blood lactate and lactate-$C^{14}$ after a single injection of a tracer amount of DL-lactate-2-$C^{14}$. Blood lactate was about 18% (at 3 min) and 11% (at 15 min) higher in diabetic rats than in normal rats. The disappearance of labelled lactate from the blood was almost the same in diabetic rats as compared with that in normal rats.

Diabetic rats expired about 12% less $CO_2$ than normal rats, but this was not significant. The amount of $C^{14}O_2$ expired by normal and diabetic rats from DL-lactate-2-$C^{14}$ is shown in Fig 2, expressed as per cent of injected dose. The mean values for the expired $C^{14}O_2$ in 4 hours was $62.3 \pm 2.6\%$ in normal and $27.6 \pm 1.8\%$ in diabetic rats. The difference in the rate of oxidation of DL-lactate-2-$C^{14}$ to $C^{14}O_2$ between the normal and diabetic rats was statistically significant ($p<0.01$) throughout the experimental period.

Table I shows that in diabetic rats, more $C^{14}$ appeared in extracellular glucose than in normal rats given DL-lactate-2-$C^{14}$. In the diabetic rats, about 4 times as much $C^{14}$ was found in the glucose-$C^{14}$ as compared to normal rats ($p<0.01$).

*Discussion.* It has been known from *in vitro* experiments that animal tissues produce only L(+) lactate(10,11). Experiments with intact animals also suggested that the natural form was preferred for energy me-

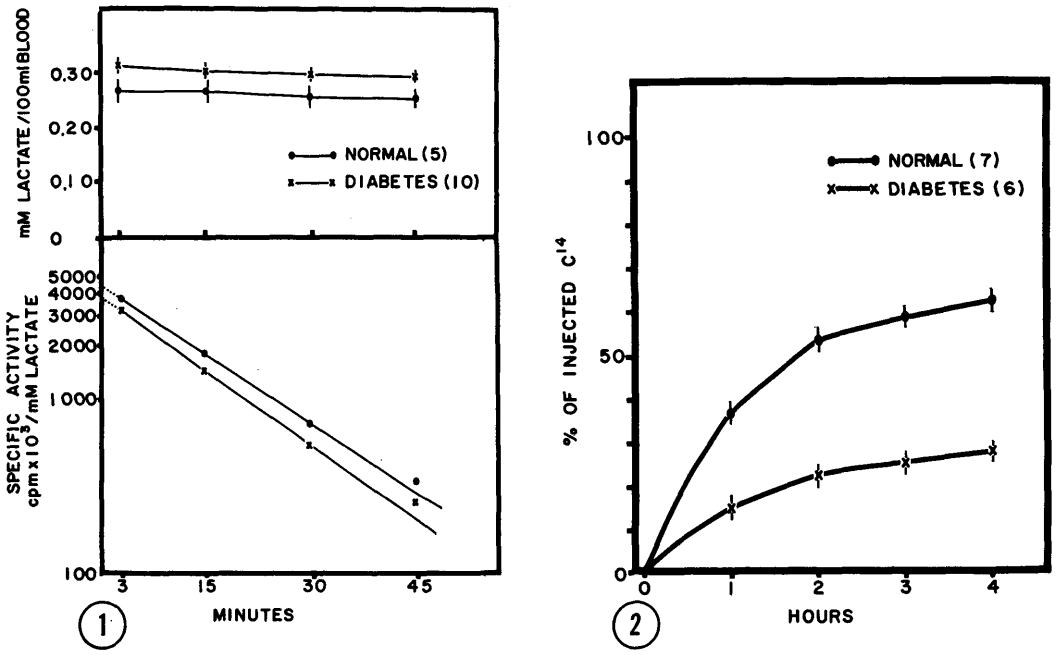


FIG. 1. Concentration of lactate and specific activity of lactate-$C^{14}$ in the blood of normal and diabetic rats after intravenous injection of DL-lactate-2-$C^{14}$. Each value represents mean ± standard error. Numbers in parenthesis indicate number of animals.

FIG. 2. Expiration of $C^{14}O_2$ by normal and diabetic rats given DL-lactate-2-$C^{14}$ intravenously, expressed as per cent of injected dose. Each value represents mean ± standard error. Numbers in parenthesis indicate number of animals.

tabolism and glycogen deposition(12,13). However, it has been established that racemic lactate is practically completely metabolized by the intact animals(14).

The present findings do not permit the actual estimations of the size of blood lactate pool and of values for turnover in the blood, and the rate of oxidation of natural lactic acid, because of the use of the racemic lactate. To obtain some information about the metabolic state of blood lactate, it is of interest even with the use of the racemic lactate to estimate the rate of removal and entry of blood lactate. Table II represents the result using an isotopic tracer procedure to determine the rate of entry and removal of lactate in normal and diabetic rats. These equations were originally applied to the calculation of turnover rate of ketone body, then applied to study the glucose removal and entry by Weinhouse(15). In this calculation, one makes the reasonable assumption that any new lactate molecules entering the blood will be predominantly unlabelled. The turnover rate of circulating blood lactate was 15.2 μM/100 ml/min in normal rats and 19.2 μM/100 ml/min in diabetic rats. Since the blood lactate was constant in both groups, it was assumed that the rates of entry and removal were equal.

TABLE I. Concentration of Glucose and Glucose-$C^{14}$ in the Blood of Normal and Diabetic Rats After Injection of DL-Lactate-2-$C^{14}$.

| | No. of exp | mM glucose/100 ml blood | | | % of injected dose/100 ml blood | |
|---|---|---|---|---|---|---|
| | | 0 hr | 2 hr | 4 hr | 2 hr | 4 hr |
| Normal | 5 | 0.569 ± 0.028* | 0.533 ± 0.033 | 0.515 ± 0.026 | 14.3 ± 2.0 | 12.7 ± 2.2 |
| Diabetes | 5 | 2.311 ± 0.446† | 2.138 ± 0.384† | 2.171 ± 0.372† | 58.6 ±10.4† | 43.2 ± 8.4† |

* Mean ± standard error. † $p < 0.01$.

TABLE II. Rate of Formation of Blood Lactate and Rate of Disappearance of Blood Lactate in Normal and Diabetic Rats Calculated from the Change in Blood Lactate-$C^{14}$.*

| Group | No. of exp | Metabolic rate of blood lactate, $\mu$M/100 ml/min: Entry into blood stream | Removal from blood stream | Net change |
|---|---|---|---|---|
| Normal | 5 | 15.2 ± .9† | 15.7 ± .7 | —.5 |
| Diabetes | 10 | 19.2 ± 1.2‡ | 19.9 ± 1.2‡ | —.7 |

* The rate is calculated from the fall in specific activity using following equations with assumption (see *Discussion*)

$$R_1 \text{ (entry)} = \frac{(B_o - B_t) \log i_o/i_t}{T \log B_o/B_t} \qquad R_2 \text{ (removal)} = \frac{(B_o - B_t)}{T} + R_1$$

where $R_1$ is rate of entry, $R_2$ is rate of removal, $B_o$ is initial lactate concentration, $B_t$ is concentration at time t, and $i_o$ and $i_t$ are initial and final specific activity of lactate. (—) is rate of decrease in blood lactate.

† Mean ± standard error. ‡ $p < .05$.

These results would indicate that the blood lactate is being renewed at a very rapid rate even in severe diabetic rats, where blood lactate concentration was usually higher than in normal rats. Shreeve *et al*(3), studying the metabolism of intravenously administered DL-lactate-2-$C^{14}$ in the human being demonstrated that the disappearance of labelled lactate from the blood was much slower in the case of the more severe diabetic patients, which suggested a mild lactic acidemia. However, they did not discuss their data from mild diabetics whose blood lactate, with the same disappearance of labelled lactate from the blood as non-diabetic subjects, was about twice the normal values.

The specific activity of the lactate administered was diluted by endogenous material depending upon the size of the pool. From the calculation of our results using racemic lactate, lactate pool size was about 12% greater in diabetic than in normal rats. Therefore, pool size was not a major factor in the decrease of expired $CO_2$ from DL-lactate-2-$C^{14}$ in alloxan diabetic rats. An increased excretion of lactate in the urine also should not be a factor for the decrease in expired $CO_2$ in diabetic rats, since Steenrod found that 95 to 100% of the radioactivity in the urine of diabetic rats appeared in glucose(5).

Whether or not the tricarboxylic acid cycle activity is decreased in diabetic animals has not been established. The decrease in $C^{14}O_2$ expiration, associated with diabetes, despite a faster disappearance rate of lactate from the blood, was accompanied by a significant increase in the $C^{14}$ content of the blood glucose. De Meutter and Shreeve(16) demonstrated an increased incorporation of lactate, pyruvate and acetate into glucose in diabetic animals. Therefore, it seems reasonable to suppose that lactate is being channeled to glucose formation rather than to oxidation at a branch point of the metabolic pathways for lactate.

It is not clear whether DL-lactate is metabolized in a normal manner by diabetic rats. Further studies are necessary using the L form of lactate. However, our present results are consistent with the indications of Steenrod that lactate is metabolized at essentially the same rate in both normal and diabetic animals but that in the diabetic animals, a greater portion of the carbon atoms of lactate is incorporated into glucose and less converted to $CO_2$.

*Summary.* The metabolism of lactate in normal and diabetic rats has been studied using a single intravenous injection of DL-lactate-2-$C^{14}$. The concentration of blood lactate was found to be about 15% higher in diabetic rats than in normal rats. The disappearance of labelled lactate from the blood was almost the same in diabetic as compared with that in normal rats. Diabetic rats showed depressed $C^{14}O_2$ production and increased incorporation of $C^{14}$ in the blood glucose from DL-lactate-2-$C^{14}$ as compared with normal rats. These results would indicate that the blood lactate is being used up and renewed at a very rapid rate even in diabetic state. However, in the diabetic state,

it would appear that the lactate is being channeled to glucose formation rather than to oxidation.

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## Transformation of Hamster Embryo Cells *in vitro* by Murine Sarcoma Virus (Harvey).* (32326)

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Harvey(1) reported the induction of tumors in a variety of rodents by an agent which was isolated from a rat inoculated with Moloney leukemia virus. Chesterman *et al*(2) described the pathology of tumors and other lesions induced in mice, rats and hamsters by the same virus.

Moloney(3) later described the isolation of a virus which induced rhabdomyosarcomas in mice. Huebner *et al*(4) have shown that the Moloney isolate is capable of inducing fibrosarcomas in hamsters which unlike the mouse tumors, do not contain infectious virus. Evidence of the presence of a defective viral genome in the hamster tumor cells was presented(4). The *in vitro* transformation of mouse embryo cells(5) and rat embryo cells (6) by the Moloney sarcoma virus and of mouse embryo cells by the Harvey virus(7) has been described.

For convenience the agents have been termed "Murine Sarcoma Viruses" (MSV) and designated MSV (Harvey) and MSV (Moloney), respectively.

This communication describes the *in vitro* transformation of hamster embryo cells by MSV (Harvey).

*Materials and methods.* Virus. The source of the MSV and the initial transformation of mouse embryo cells has been described(7).

*Cell culture techniques.* Hamster embryo cell cultures were prepared and maintained by the same methods as previously described for mouse embryo cells(7). MSV preparations were assayed by determining the number of foci of transformed cells produced in cultures of Swiss T-O mouse embryo cells. Five hundred thousand mouse embryo cells per

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