regulation of energy metabolism, still remains to be found. Steroids may be involved in the energy utilizing steps by which amino acids are converted to proteins. They may affect the oxidation of some essential amino acid and indirectly alter the protein synthesis(16). It may be that the primary effect of steroids is on cellular permeability. It cannot be concluded from this study that the above mentioned antagonism in enzymatic effects is related to the catabolic or anabolic activity of the steroids studied. Further work may show that such a relationship does occur.

Summary. The activities of 2 mitochondrial respiratory enzymes were determined in the liver of normal rats and of rats treated with cortisone and/or anabolic steroid Methandrostenolone. The activities of malic dehydrogenase and DPN-cytochrome-c-reductase were decreased in cortisone treated rats. This depression was reversible and interruption of treatment resulted in normalization of activities of both enzymes. Simultaneous treatment of rats by cortisone and the anabolizer did not, however, prevent this depression. The activities of both enzymes were increased in rats treated with Methandrostenolone alone.

1. Kowalewski, K., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 971.

2. Kowalewski, K., Bekesi, G., *ibid.*, 1961, v106, 300.

3. ———, *Arch. intern. pharmacod. et Ther.*, 1962, v135, 393.

4. Schneider, W. C., van Potter, R., *J. Biol. Chem.*, 1949, v177, 893.

5. Cleland, K. W., Slater, E. C., *Biochem. J.*, 1953, v53, 547.

6. Kowalewski, K., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 448.

7. ———, *Endocrinology*, 1958, v62, 493.

8. Schneider, W. C., *J. Biol. Chem.*, 1948, v176, 259.

9. Schneider, W. C., Hogeboom, G. H., *ibid.*, 1950, v183, 123.

10. Umbreit, W. W., Burris, R. H., Stauffer, J. F., *Manometric Techniques*, Burgess Publ., 3rd ed., 1959, p177.

11. Lacroix, E., Leusen, I., *Acta Endocrin.*, 1959, v31, 324.

12. Lacroix, E., *Arch. Intern. Physiol. et Bioch.*, 1959, v67, 539.

13. Smith, A. L., Koeppe, O. J., Franz, J. M., *Endocrinology*, 1961, v69, 872.

14. Kerppola, W., *ibid.*, 1960, v67, 252.

15. Kowalewski, K., in *Ciba International Symposium on Protein Metabolism*, Springer Verl., 1962, p237.

16. Lardy, H. A., in *Hormonal Regulation of Energy Metabolism*, Ed. by L. W. Kinsell, C. C Thomas, U.S.A., Publ., 1957, p45.

Received March 15, 1963. P.S.E.B.M., 1963, v113.

Comparative Studies in Rous Sarcoma. IV. Glucose Metabolism of Normal and Rous Sarcoma Virus-Infected Cells.* (28351)

HERBERT R. MORGAN AND SEETHA GANAPATHY

Louis A. Wehle Virus Research Laboratory, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Previous studies have shown that chick embryo fibroblasts infected with Rous sarcoma virus (RSV) *in vitro* acquire certain new morphologic, growth and synthetic properties(1-8) in which they resemble the malignant cells found in Rous sarcomas. It has been observed that when chick embryo cells are infected with RSV *in vitro*, the cultures accumulate lactic acid(2) and it has been assumed that this was due to an increase in the aerobic glycolytic utilization of glucose by the virus-infected cells. This metabolic pattern for glucose utilization has been accepted as one of the characteristics of cancer cells *in vivo*(9) and is exhibited by Rous sarcoma tumor tissue(10) and by chorioallantoic membrane cells infected with RSV *in vivo*(11). It was of interest, therefore, to

* Supported by grants from Nat. Cancer Inst., USPHS, from the N. B. Delavan Virus Research Fund, and from the United Funds of Clayton and Alexandria Bay, N. Y.

compare the metabolic activities of normal chick embryo cells and aliquots of these cells infected with RSV maintained under the same conditions *in vitro* to see if the virus-infected cells would develop this increase in aerobic glycolytic activity.

Materials and methods. The Rous sarcoma virus employed was derived from the standard strain (batch CT776) of Dr. W. R. Bryan of the Nat. Cancer Inst., and partially purified virus stock (RSV) was prepared according to the method of Moloney(12). These virus preparations were assayed by the method of Temin and Rubin(4) employing chick embryo cell monolayers, and the results recorded as focus-forming units (FFU).

A medium composed of 8 parts Eagle's medium with double the concentration of amino acids and vitamins, 1 part Difco bacto-tryptose phosphate broth, and 1 part chicken or calf serum or dialyzed calf serum was used(4).

Ten-day-old chick embryos of a suitable strain of susceptible white Leghorn chickens were used to prepare primary cultures in Petri dishes by the methods described(4). After one subculture, one-half of these cells were infected with RSV (2×10^5 FFU per Petri dish) in the medium containing 10% calf serum in a CO_2-air flow incubator, with the remainder serving as uninfected controls. The cells were subcultured twice and examined to determine that most of the cells gave visible evidence of being transformed by the virus.

The cells both infected and control were removed from the Petri dishes with 0.05% trypsin in tris buffered saline, sedimented in a centrifuge and resuspended in medium containing 1% dialyzed calf serum to which varying amounts of glucose had been added. The number of cells in the suspension was determined by counting in a hemocytometer, adjusted to contain 10×10^6 cells per ml, and the number of infected cells determined by the infective center assay method(4). One ml of the infected or control cell suspensions was introduced into Warburg flasks, each with 0.2 ml of 20% KOH in the center well. The flasks were placed in the Warburg water bath at 37°C and equilibrated for 10 minutes. Manometer readings were made every hour for 4 hours. At the conclusion of the experiment the fluids were removed from each flask and cells removed by sedimentation in the centrifuge. The supernatant fluids and aliquots of the original suspending medium were then assayed for content of glucose by the micromethod described by Gradwohl(13) and content of lactic acid by the method of Barker and Summerson (14), and glucose utilization and lactic acid production determined.

At the time the cells were harvested for the respiration measurements, the content of lactic dehydrogenase in the medium removed from the cultures was determined by the method described by Kelley(15) employing the reagents described(16) and the lactic dehydrogenase contents expressed as B-B (Berger-Broida) units(16).

Results. The results of representative experiments, using 2 different concentrations of glucose, are presented in Table I. These data show that with a given quantity of glucose in the medium, the oxygen consumption of chick embryo cells is reduced when they are infected with RSV and their utilization of glucose and production of lactic acid are increased. This indicates that the aerobic glycolytic activity of such infected cells is increased and this is associated with an increase in amount of the enzyme lactic dehydrogenase which can be detected in the tissue culture fluids bathing such cells.

Discussion. Our results with normal chick embryo cells infected with RSV reveal that in their reduced consumption of oxygen, increased utilization of glucose and increased production of lactic acid under aerobic conditions, infection with the virus causes metabolic changes which are characteristic of many malignant cells(9). It is apparent that more lactic acid was produced during this period of 4 hours than could be accounted for by the utilization of glucose in the freshly added culture medium, and it was probably derived from the nutrients present in the cells prior to their suspension in the

TABLE I. Oxygen Consumption, Glucose Utilization, Lactic Acid Production and Lactic Dehydrogenase Production by Cells.

Exp	Cells	Initial glucose conc, μmoles/ml	Oxygen consumption,* μl of O_2	Glucose utilization,* μmoles	Lactic acid production,* μmoles	Lactic dehydrogenase in culture medium,† B-B units/ml
A	Uninfected	5.5	6.8	0.38	0.86	250
	Infected‡	5.5	2.3	0.50	1.42	425
B	Uninfected	9.5	5.4	0.49	1.03	775
	Infected	9.5	2.7	0.93	2.06	1,225

* Values are expressed per 10^6 cells in 4-hr period of experiment.
† Values are expressed per ml per 10^6 cells in 24-hr period preceding harvest of cells.
‡ Infective center assays revealed that 70% of cells were infected.

fresh medium for the respiration experiments. The metabolic changes induced in these cells by infection with RSV *in vitro* are similar to those produced by RSV infection *in vivo* of the chorioallantoic membrane tissues of the chick embryo as described by Levine, Ashmore and associates(11,17,18). They showed that infection with RSV caused a decrease in the oxygen consumption of membrane fragments(11), an increase in utilization of glucose(11,17) and an increase in lactic acid production(17) associated with an increase in content of the infected cells in lactic dehydrogenase and other glycolytic enzymes(18) which indicated a preferential synthesis of these enzymes in the virus-induced tumor cells. The observations reported here and the studies of Levine, Ashmore and associates(11,17,18) are in agreement with the high glycolytic rate demonstrated earlier in the Rous sarcoma tissue by Burk *et al.*(10). Thus, in this simple *in vitro* system a large part of a population of normal chick embryo cells can be transformed by infection with RSV and subsequently exhibit morphological, growth and synthetic activities characteristic of malignant cells found in Rous sarcomas(1-5) and increased aerobic glycolytic activity which is not only characteristic of Rous sarcoma cells but also of many other malignant cells (9). This experimental system obviously possesses advantages for such studies of the metabolic changes accompanying malignant transformation, for it permits direct comparison of elements of a single population of cells maintained under controlled conditions *in vitro* when one sample of this population is undergoing malignant transformation induced by the RSV.

Summary. Chick embryo fibroblasts infected *in vitro* with RSV exhibit striking alterations in their metabolism which include a decrease in oxygen consumption, increase in glucose utilization and lactic acid production, and an increase in release of lactic dehydrogenase into the culture fluids under aerobic conditions. These changes are associated with the increased aerobic glycolytic activity of these cells which is characteristic of Rous sarcoma and other neoplastic cells.

The authors are indebted to Angelo P. Andrese and Lorraine A. Lorenz for valuable technical assistance.

1. Manaker, R. A., Groupé, V., *Virology,* 1956, v2, 838.
2. Rubin, H., *ibid.,* 1960, v10, 29.
3. Erichsen, S., Eng, J., Morgan, H. R., *J. Exp. Med.,* 1961, v114, 435.
4. Temin, H. M., Rubin, H., *Virology,* 1958, v6, 669.
5. ———, *ibid.,* 1959, v8, 209.
6. Dougherty, R. M., Morgan, H. R., *J. Exp. Med.,* 1962, v115, 245.
7. Morgan, H. R., Andrese, A. P., *ibid.,* 1962, v116, 329.
8. Pontén, J., *J. Nat. Cancer Inst.,* 1962, v29, 1147.
9. Boxer, G. E., Devlin, T. M., *Science,* 1961, v134, 1495.
10. Burk, D., Sprince, H., Spangler, J. M., Kabat, E. A., Furth, J., Claude, A., *J. Nat. Cancer Inst.,* 1941, v2, 201.
11. Levine, A. S., Stricker, F., Uhl, R., Ashmore, J., *Nature,* 1960, v188, 229.
12. Moloney, J. B., *J. Nat. Cancer Inst.,* 1956, v16, 877.
13. Gradwohl, R. B. H., in *Clinical Laboratory*

Methods and Diagnosis, Volume 1, The C. V. Mosby Co., St. Louis, 1943.

14. Barker, S. B., Summerson, D. H., *J. Biol. Chem.,* 1941, v138, 535.

15. Kelley, R., Greiff, D., *J. Exp. Med.,* 1961, v113, 125.

16. Berger, L., Broida, D., *Sigma Tech. Bull.,* 1960, No. 500.

17. Levine, A. S., Uhl, R., Ashmore, J., *J. Nat. Cancer Inst.,* 1961, v27, 597.

19. Weber, G., Banerjee, G., Levine, A. S., Ashmore, J., *ibid.,* 1961, v27, 869.

Received April 1, 1963. P.S.E.B.M., 1963, v113.

Changes in Heart Rate on Intravenous Infusion in Dogs with Chronic Cardiac Denervation.* (28352)

DAVID E. DONALD AND JOHN T. SHEPHERD

Sections of Surgical Research and of Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minn.

It was found previously that when dogs with chronic cardiac denervation were made to exercise on a treadmill, there was a characteristic pattern of change in heart rate: a slow increase on starting to run, a maximum reached after 1½ minutes, and a slow decrease when the exercise was stopped. The maximal heart rates were proportional to the grade of work; at severe levels of work, as represented by an oxygen consumption of 65 ml/kg/min, the rate increment was 50 to 60 beats more than the pre-exercise value(1). It was found that this increase in heart rate was not affected by bilateral adrenalectomy nor was it related to the absolute value of blood temperature during the exercise(2). Studies by Blinks(3) on a number of animal species showed that distention of the right atrium in isolated heart preparations had a positive chronotropic effect. It seemed appropriate to examine the possibility that this mechanism was responsible for the increase in heart rate seen during exercise in dogs with chronic cardiac denervation.

Either 5% dextrose in physiologic saline or 6% dextran (molecular weight 40,000) at body temperature was infused intravenously into dogs in which chronic cardiac denervation previously had been performed by the neural ablation technic of Cooper and associates(4). Studies were carried out on 6 animals with proven cardiac denervation(4); 2 animals were conscious and 4 were anesthetized. In the conscious dogs, only the volume and rate of fluid infused and the heart rate were recorded. In the dogs anesthetized with sodium pentobarbital (20 mg/kg), the following were recorded: temperature and pressure of the blood within the right atrium, heart rate, esophageal pressure at the level of the atrium, and volume and rate of infusion. Prior to the time of study, bilateral adrenalectomy had been carried out in 2 animals.

Two animals were studied twice on separate occasions, once using dextrose saline and again using dextran. The data for the group are summarized in Table I. The time course of events during an infusion of dextrose saline is shown in Fig. 1, and during an infusion of dextran in Fig. 2.

Discussion and conclusions. In all cases, an increase in heart rate resulted from intravenous infusion of fluid into dogs with chronic cardiac denervation, the increments ranging from 4 to 31 beats per minute. In those cases in which it was measured, right atrial pressure increased; in individual experiments, the increase in pressure was proportional to the volume infused. In the experiments with dextran, the maximal increase in right atrial pressure preceded the maximal increase in heart rate by intervals ranging from 4 to 54 minutes. Part of this wide variation was explained by the fact that the peak of the increase in rate often was approached so slowly that it was difficult to

* This investigation was supported in part by research grant from Nat. Inst. Health, P.H.S.