process would appear to be a dominant factor in determining over-all strontium calcium relationships.

The advice of Dr. M. F. Sullivan in setting up the perfusion apparatus and the technical assistance of Mrs. Joan Hess are gratefully acknowledged.

1. Wilson, T. H., Wiseman, G., *J. Physiol.*, 1954, v123, 116.
2. Schachter, D., Rosen, S. M., *Am. J. Physiol.*, 1959, v196, 357.
3. Schachter, D., Dowdle, E. G., Schenker, H., *ibid.*, 1960, v198, 263.
4. Dowdle, E. B., Schachter, D., Schenker, H., *ibid.*, 1960, v198, 269.
5. Wasserman, R. H., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 92.
6. Curran, P. F., Solomon, A. K., *J. Gen. Physiol.*, 1957, v41, 143.
7. Dumont, P. A., Curran, P. F., Solomon, A. K., *ibid.*, 1960, v43, 1119.
8. Wasserman, R. H., Kallfelz, F. A., Comar, C. L., *Science*, 1961, v133, 883.
9. Harrison, H. E., Harrison, H. C., *Am. J. Physiol.*, 1960, v199, 265.
10. Palmer, R. F., Thompson, R. C., Kornberg, H. A., *Science*, 1958, v127, 1505.
11. Thompson, R. C., Palmer, R. F., *Am. J. Physiol.*, 1960, v199, 94.
12. Cramer, C. F., Copp, D. H., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 514.

Received July 5, 1961. P.S.E.B.M., 1961, v108.

Coenzyme Q Concentration in Proliferative Lesions of Liver.* (26920)

KENNETH A. OSTERBERG AND LEE W. WATTENBERG
Department of Pathology, University of Minnesota School of Medicine, Minneapolis

Histochemical studies of the succinic dehydrogenase system using tetrazolium salt reduction have shown a decrease of succinate-tetrazolium reductase activity in a number of benign and malignant proliferative lesions (1,2,3). Recent work employing the tetrazolium salt, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), has indicated that a member of the coenzyme Q group of quinones serves as an intermediate electron transport agent in the succinate-INT reductase reaction, and that in many tissues this system is not saturated with respect to quinone(4,5,6). Accordingly, a decrease in succinate-INT reductase activity might be due to either a decrease in activity of the primary dehydrogenase, a lack of availability of quinone, or some combination of the two effects. An effort was made to ascertain which of these alternatives exists in regenerating liver and the Novikoff hepatoma by addition of coenzyme Q_{10} to the reaction mixture. It was found that with frozen sections of the Novikoff hepatoma addition of coenzyme Q_{10} to the succinate-INT system resulted in a considerably greater enhancement of reductase activity than occurs in normal liver(5). This suggested that the coenzyme Q content of this neoplasm might be relatively low. In contrast, in regenerating liver addition of coenzyme Q_{10} to the reaction mixture caused an enhancement of activity of approximately the same order of magnitude as that of normal liver. These findings suggested that the coenzyme Q content of regenerating liver was not significantly reduced, but that some other factor was causing the decrease in activity observed. Since the enhancement of activity of the succinate-INT reductase activity by addition of coenzyme Q_{10} to the reaction mixture can be considered at best as supplying only indirect evidence of the relative amount of coenzyme Q available for the reductase reaction, direct quantitative studies of the coenzyme Q content of these tissues were undertaken. The results clearly demonstrate that the Novikoff hepatoma and the more slowly growing Morris hepatoma #5123 have a considerably lower concentration of coenzyme Q than normal liver, while in regenerating liver the coenzyme Q concen-

* This investigation was supported by grant B-2566 from Nat. Inst. of Neurol. Dis. and Blindness, N.I.H., U.S.P.H.S., Bethesda, Md.

TABLE I. Tissue Concentration of Coenzyme Q.

Type of tissue	Determinations	CoQ concentration*
Normal liver	8	611 ± 144
48 hr sham-operated control	5	765 ± 166
72 hr " " "	3	658 ± 173
48 hr regenerating liver	5	705 ± 69
72 hr " "	3	602 ± 70
Morris hepatoma	6	211 ± 53
Novikoff "	4	325 ± 87
Heart	2	958 ± 31
Kidney	2	419 ± 85
Spleen	2	212 ± 12

* μg/g dry wt as CoQ_{10} (mean ± stand. dev.).

tration is comparable to that of the normal liver.

Materials and methods. Quantitative determination of coenzyme Q was carried out by a modification of the method of Crane *et al.*(7). Weighed amounts of tissue were homogenized in saline and refluxed for 30 minutes in an equal volume of 15% KOH in methanol containing pyrogallol. Non-saponifiable lipids were extracted with isooctane. The isooctane extracts were washed, then purified on a 2 × 3 cm Decalso (50/80 mesh) chromatographic column. The coenzyme Q fraction, eluted with 8% (v/v) ethyl ether in isooctane, was evaporated to dryness *in vacuo* and dissolved in absolute ethanol. Coenzyme Q was determined by measuring optical density at 275 mμ before and after reduction with KBH_4. Total coenzyme Q was determined as coenzyme Q_{10}, utilizing $\Delta E^{1\%}_{1\,cm}$ (oxidized-reduced) 275 mμ = 142. Quantitative histochemical procedures for succinate-INT reductase are the same as those used previously(4). Female Sprague-Dawley rats 3-4 months of age obtained from the Holtzman Co. were used throughout, except for work on the Morris Hepatoma 5123.† In hepatic regeneration experiments approximately two-thirds of the liver was excised (8). The Novikoff hepatoma was grown intraperitoneally as a solid tumor(9). Tumors 5 or 6 days following transplantation were used.

† The Morris Hepatoma was grown in Buffalo strain rats. We wish to thank Dr. Harold P. Morris, Nat. Cancer Inst., for supplying us with tumor bearing animals.

Results. The results are summarized in Tables I and II. The coenzyme Q concentration of both the Morris hepatoma and the Novikoff hepatoma is considerably lower than that of normal liver, and in these tissues addition of coenzyme Q_{10} to the succinate-INT reductase reaction results in considerably greater enhancement of activity than is found with the normal liver. Regenerating liver has a coenzyme Q concentration similar to that of normal liver, and enhancement of succinate-INT reductase activity by addition of coenzyme Q is also of a similar order of magnitude to that of normal liver.

Heart, kidney, and spleen from normal rats are included in the present study to show a range of succinate-INT reductase activities and coenzyme Q concentrations in several normal tissues. It will be noted that spleen bears a considerable resemblance to the 2 hepatomas studied in that succinate-INT reductase activity is low, addition of coenzyme Q_{10} causes a large enhancement in this activity, and coenzyme Q concentration of the tissue is of the same order of magnitude as that of the 2 malignant tissues.

Discussion. These results have demonstrated that there is no significant change

TABLE II. Effect of Coenzyme Q_{10} Addition on Succinate-INT Reductase Activity.

Type of tissue*	Succinate-INT reductase activity† No addition	Succinate-INT reductase activity† CoQ_{10} added	% increase enzyme activity
Normal liver (2)	179 ± 9	235 ± 7	31
48 hr sham-operated control (3)	198 ± 14	288 ± 25	45
72 hr sham-operated control (3)	177 ± 26	228 ± 38	33
48 hr regenerating liver (3)	96 ± 13	143 ± 24	48
74 hr regenerating liver (3)	147 ± 30	191 ± 25	30
Morris hepatoma (4)	118 ± 12	239 ± 24	103
Novikoff hepatoma (2)	29 ± 1	65 ± .4	117
Heart (2)	362 ± 4	392 ± 39	8
Kidney (2)	297 ± 6	341 ± 13	15
Spleen (2)	69 ± .1	169 ± 7	145

* No. of determinations in parentheses.
† 1000 × ΔO.D. at 490 mμ/mg dry wt/min. (mean ± stand. dev.).

in tissue concentration of coenzyme Q during liver regeneration, and suggest that relatively rapid coenzyme synthesis takes place in this tissue. High levels of coenzyme are maintained at a time when the cell is under the stringent metabolic demands occasioned by its own reproduction. The low reductase activity can be accounted for by a lack of primary dehydrogenase or by an inability of newly forming dehydrogenase to couple with the adequate amounts of coenzyme Q present. In support of this latter possibility is the observation that menadione causes a relatively greater degree of enhancement of succinate-INT reductase activity in regenerating liver than in normal liver(5).

There is additional support for the concept that endogenous coenzyme Q does not function optimally in the succinate-INT reduction system of sections of regenerating liver. It has been shown(6) that sections of liver from normal rats injected with coenzyme Q_{10} show an enhanced reductase activity and a marked decrease in ability of additional coenzyme Q_{10} to increase the succinate-INT reductase activity when subsequently added *in vitro*(6). These observations indicate that the degree of unsaturation of the succinate-INT reductase system can be altered by changes in amount of coenzyme Q_{10} available to liver cells *in vivo*. In the present study it has been found that the amount of coenzyme Q relative to the succinate-INT reductase activity is considerably higher in regenerating liver than in normal liver. Therefore, it would be expected that the degree of enhancement of reductase activity obtained by addition of coenzyme Q_{10} would be less in regenerating liver than in normal liver. That this is not the case suggests that in regenerating liver intracellular sequestration of the coenzyme Q takes place.

In both the Morris hepatoma and the Novikoff hepatoma a low concentration of coenzyme Q has been found. Both hepatomas likewise have succinate-INT reductase systems whose activities can be markedly enhanced by addition of coenzyme Q_{10}. These findings suggest that in the 2 neoplasms a non-availability of optimal amounts of coenzyme Q is an important factor in the low succinate-INT reductase activities observed. In the Novikoff hepatoma a second factor appears to participate. In this tumor a high ratio of coenzyme Q to succinate-INT reductase activity occurs suggesting that the limited amount of coenzyme Q present does not maximally function in the reductase reaction. Thus there would appear to be either a deficiency in primary dehydrogenase or an inability of the primary dehydrogenase present to couple with the available coenzyme Q. During the 6 months preceding this study a striking decrease, almost 50%, in succinate-INT reductase activity of the Novikoff hepatoma was observed. Ability of coenzyme Q_{10} to enhance the reductase activity also diminished. This tumor tends to become necrotic quite rapidly and while morphologically non-necrotic portions were used for the analytic studies reported it is possible that some of the changes observed are due to inclusion of cells which were undergoing incipient degenerative changes.

The studies of normal tissues have shown that there is a similarity between concentration of coenzyme Q in spleen and the 2 hepatomas and, in addition, that the succinate-INT reductase activity of spleen is as markedly enhanced by *in vitro* addition of coenzyme Q_{10} as is that of the hepatomas. These data provide evidence that the hepatomas do not have an actual deficiency of quinone. Whether or not the pattern of low coenzyme Q concentration and marked unsaturation of the succinate-INT reductase system with respect to quinone has some functional significance remains to be determined.

Conclusion. Quantitative studies of normal and regenerating liver have shown that high concentrations of coenzyme Q are maintained during the proliferative process and indicate that a lack of coenzyme is not responsible for the low succinate-INT reductase activity observed during regeneration. In contrast, a low coenzyme Q concentration in the Morris hepatoma and the Novikoff hepatoma appears to play an important role in the low reductase activities observed in these 2 neoplasms.

1. Wattenberg, L. W., *Cancer Res.*, 1959, v19, 1118.

2. Ogawa, K., Zimmerman, H. M., *J. Histochem. Cytochem.*, 1959, v7, 342.

3. Monis, B., Nachlas, M. M., Seligman, A. M., *Cancer*, 1959, v12, 1238.

4. Wattenberg, L. W., Leong, J. L., *J. Histochem. Cytochem.*, 1960, v8, 296.

5. Wattenberg, L. W., Gronvall, J. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 394.

6. Wattenberg, L. W., *Ciba Foundation Symposium on Quinones in Electron Transport*, J. and A. Churchill Ltd., London, 1961, p. 367.

7. Crane, F. L., Lester, R. L., Widmer, C., Hatefi, Y., *Biochim. Biophys. Acta*, 1959, v32, 73.

8. Higgins, G. M., Anderson, R. M., *Arch. Path.*, 1931, v12, 186.

9. Novikoff, A. B., *Cancer Res.*, 1957, v17, 1010.

Received August 7, 1961. P.S.E.B.M., 1961, v108.

Induction by Sendai Virus of Non-transmissible Cytopathic Changes Associated with Rapid and Marked Production of Interferon.* (26921)

ION GRESSER** (Introduced by J. F. Enders)

Research Division of Infectious Diseases, Children's Hospital Medical Center, Department of Bacteriology and Immunology and Department of Medicine, Harvard Medical School, Boston

During experiments with various myxoviruses it was noted that high concentrations of chick adapted Sendai virus induced non-transmissible cytopathic effects (NTCE) in cultures of primary human amnion (HA) cells. Several observations suggested that Sendai virus underwent an incomplete cycle of multiplication in these cells, which was associated with the early liberation of large quantities of an interferon-like substance into the culture medium. These observations are described together with experiments suggesting that these phenomena may be interdependent.

Methods and materials. Cell culture. Preparation and maintenance of human amnion (HA) cells has been previously described (1). Primary cultures of rhesus monkey kidney cells were obtained commercially.†

Viruses. Relevant data on viruses employed are as follows:

Sendai virus (F. Davenport) allantois of embryonated egg 27

Sindbis virus (R. Taylor Ar-339) HA cells 10

Poliovirus II MEF chick embryo cell cultures 143, HA cells 2

Mumps virus HA cells 7

Simian virus 5, monkey kidney cells 4

DA virus‡ (P. Isaacson Rh 76) HA cells 3

Preparation of stock sendai virus. Sendai virus was prepared in 9-11 day embryonated eggs by inoculation intrallantoically of 0.1 ml of a 10^{-3} dilution of stock virus. After 72 hours incubation at 35-37°C, allantoic fluid was harvested, centrifuged for 10 minutes at 400 g, and the supernate centrifuged at 4°C for 2 hours at 110,000 g (Spinco L preparative centrifuge #40 rotor). The sediment was resuspended in a mixture of 50% tissue culture medium and 50% skimmed milk and stored at −70°C.

Virus assay. Infectivity titers were calculated by the formula of Reed and Muench. Sendai virus was titrated in embryonated eggs (EID_{50}) taking as criterion of infection appearance of hemagglutinin in allantoic fluids. Sindbis, poliovirus, DA, SV 5 and mumps viruses were prepared and titrated in HA cells. In this system more than 25% cell destruction was considered evidence of viral multiplication or NTCE in the case of Sendai virus. Hemagglutination and hemadsorption tests were performed by standard methods(2,3).

* Supported in part by Research Grant from U. S. Public Health Service, Dept. of HEW.

** Postdoctoral Research Fellow of National Foundation.

† Microbiological Associates, Inc., Washington, D. C.

‡ A myxovirus isolated from the blood of a patient with viral hepatitis. This virus is considered closely related if not identical to SV 5 and SA viruses.