encephalographic changes were noted in every patient studied, paralleling the clinical alterations. The earliest changes, generally occurring within 24 to 48 hours after the beginning of treatment, consisted of low-voltage fast activity (20 cy/sec), particularly prominent in the frontal regions; this was the sort commonly encountered with a variety of drugs, such as barbiturates. Increasing slowing in all leads was then observed, with abolition of the alpha rhythm; these changes became maximal on the day of most striking hallucinations. The degree of slowing varied from patient to patient; a frequency of 4 to 5 cy/sec was common, and occasionally waves of 1 to 2 cy/sec were encountered. The slow waves were of moderate to high voltage, and tended to occur in episodic bursts simultaneously in all leads. The focal pattern noted in one patient prior to therapy disappeared, being replaced by the typical abnormality observed in the others. With clinical recovery, the electroencephalographic abnormalities subsided, and the pattern became normal after several days.

Five patients died during or soon after the completion of therapy. In one of these it was possible to study the brain pathologically; no significant alterations were found.

The clinical and electroencephalographic observations indicate that the abnormalities are reversible, presumably drug-induced changes; the absence of meaningful morphologic changes in the one brain studied pathologically supports this contention. The fact that after institution of therapy with dimethylacetamide a period of a few days always elapsed before these abnormalities appeared suggests that these effects are not due to a direct toxic effect of the compound per se, but rather that by metabolic processes as yet undetermined a different substance, capable of adversely affecting neuronal function, is formed.

ARTHUR J. WEISS, ELLIOTT L. MANCALL, JOHN A. KOLTES, JOSEPH C. WHITE, LAIRD G. JACKSON
Jefferson Medical College Hospital, Philadelphia, Pennsylvania

References and Notes

1. Unpublished observations, John L. Smith Memorial for Cancer Research, Chas. Pfizer and Co., Inc., Maywood, N.J.
2. The dimethylacetamide used in this study was supplied by the John L. Smith Memorial for Cancer Research, Chas. Pfizer and Co., Inc., Maywood, N.J.

30 October 1961

Appearance of Radioactivity in Mouse Cells after Administration of Labeled Macromolecular RNA

Abstract. Although various induced effects have been reported to follow administration of ribonucleic acid, direct evidence of cellular uptake has been lacking. Radioautographic evidence is presented of incorporation of label from radioactive macromolecular RNA presented to normal and neoplastic mouse cells. Although most of the label appeared in cellular RNA, deoxyribonucleic acid also was occasionally labeled. This suggested that at least partial degradation of the RNA occurred prior to or after incorporation.

Various functional and morphological alterations in vertebrate systems have been observed following administration of intact ribonucleic acid (RNA). These included induced protein synthesis in the adult rat (*1*), embryonic differentiation (*2*), alterations in production of antibody (*3*), recovery following lethal irradiation (*4*), and reversal of neoplastic traits (*5, 6*).

While the induced biological effects of administered nucleic acids have been known for some time, only recently has interest turned to the demonstration of cellular uptake of these materials. Incorporation of deoxyribonucleic acid (DNA) by mammalian cells has been reported by a number of authors (*7–9*). Evidence suggesting the uptake of RNA has been published by Ficq (*10*), who found considerable radioactivity in neural tubes induced by grafting an organizer containing labeled RNA to normal amphibian gastrulae. Most recently, Amos (*11*) and Niu *et al.* (*6*), through the use of counting techniques, have reported the uptake of RNA by cells in tissue culture. Our work was designed to test and localize the incorporation of normal and neoplastic RNA by various cells of the mouse in vivo and in vitro.

Labeled, macromolecular RNA was isolated from the livers of three newborn Ajax mice by the phenol method of Kirby (*12*). Each mouse was labeled over an 11-day period with a total of 135 μc of H^3-cytidine (*13*) given in twice daily intraperitoneal injections. After isolation, the protein-free RNA was incubated with deoxyribonuclease (*14*) for 2 hours to insure removal of DNA. The digest was then dialyzed overnight against normal saline, after which the RNA was reprecipitated from a 4-percent potassium acetate solution with one volume of 2-ethoxyethanol. After resuspension in normal saline, analyses of DNA and RNA content were performed with the indole (*15*) and orcinol (*16*) methods, respectively.

The neoplastic RNA was prepared by identical chemical procedures from an isologous, ascites tumor of the Ajax mouse, Sarcoma I (Bar Harbor, Maine). During a 5-day period this mouse received three intraperitoneal injections per day of a saline mixture of H^3-cytidine (*13*), H^3-uridine (*17*), and H^3-adenine (*18*), in a ratio of 2 : 1 : 1 and totaling 200 μc of radioactivity.

The ability of both normal and malignant mouse cells to utilize RNA was investigated. Sarcoma I was employed as the neoplastic cell type, while peritoneal mononuclear cells were used as a normal population. Those experiments done in vitro were performed in a Dubnoff shaking incubator at 35°C. Culture medium was No. 199 (Microbiological Associates, Bethesda, Md.), to every milliliter of which 0.3 mg of streptomycin and 70 units of penicillin G sulfate were added.

In order to investigate the uptake of normal RNA, Ajax mice with and without Sarcoma I received an intraperitoneal injection of 680 μg of labeled liver RNA in 1.0 ml of normal saline. At the same time 680 μg of this RNA was added to 1.1×10^7 sarcoma cells in 6.0 ml of medium for in vitro culture. Radioautographs (*19*) were prepared from smears of periodic samples of the sarcoma in vivo and in vitro and of aspirates of the peritoneal cavity of normal, non-tumor-bearing animals. Prior to processing, one smear of each interval was digested with ribonuclease (*20*) for 2 hours. The enzyme-treated smears with the other experimental slides were carried together through radioautography. After exposure for 20 to 26 days the preparations were developed, dried, and stained as previously described (*19*).

The uptake of neoplastic RNA was studied in vivo by injecting 170 μg of labeled sarcoma RNA intraperitoneally

Table 1. Sequential labeling of sarcoma I after in vivo presentation of macromolecular, mouse liver RNA. Range of label: ++ (greatest) through + and ± to − (least).

Time (hr)	Degree of labeling		
	Nucleolus	Nucleus	Cytoplasm
2	++	++	±
9	+	++	+
18	−	+	+

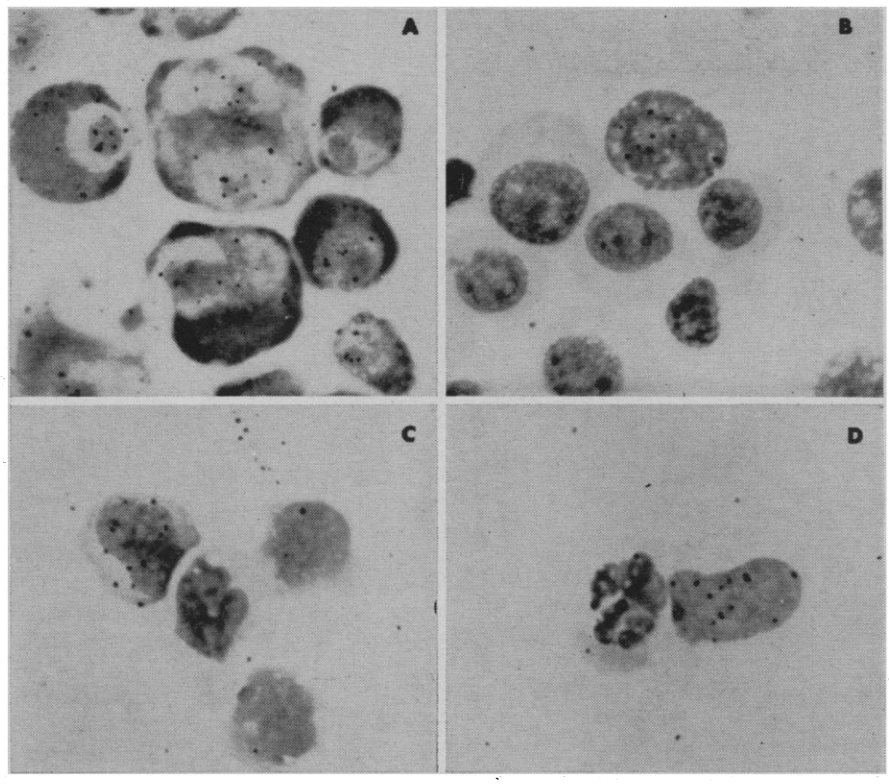

Fig. 1. Radioautographs of cells administered H^3-cytidine labeled isologous mouse liver RNA in vivo. *A*, Labeled sarcoma I cells 2 hours after administration of RNA. Note nucleolar labeling. *B*, Sarcoma cells from the same interval as *A*, showing persistent nuclear label after removal of RNA by ribonuclease treatment. *C*, Labeled normal peritoneal mononuclear cell 48 hours after administration of RNA. Note nuclear and cytoplasmic label. *D*, Peritoneal cell of the same interval as *C*, showing persistent nuclear label after removal of RNA by ribonuclease.

in tumor-bearing mice, and in vitro by adding 170 μg of this RNA to 6.0 ml of culture medium containing 1.8×10^7 sarcoma cells. Periodic smears were made for radioautography from both experiments as described above.

From Table 1 and Fig. 1*A* it is seen that sarcoma cells in vivo quickly incorporated label from the radioactive, isologous liver RNA. At the earliest interval examined (2 hours) labeling was maximal over nucleoli and nuclei. Subsequently label decreased over the nucleoli and appeared in the cytoplasm. Labeled cells were observed for as long as 48 hours after RNA injection. Appearance time and distribution of radioactivity was similar in both in vivo and in vitro experiments. However, the continuous availability of labeled RNA in vitro caused increasing amounts of label to appear with time on cultured cells. The same pattern of labeling was observed on sarcoma cells administered sarcoma RNA in vivo or in vitro.

Normal, peritoneal mononuclear cells rapidly incorporated label from radioactive liver RNA and demonstrated the same temporal labeling sequence as observed in sarcoma cells (Fig. 1*C*). Some of the labeled cells appeared to be macrophages, while others morphologically resembled undifferentiated hemopoietic cells. Large and medium peritoneal lymphocytes were frequently labeled, and, at longer intervals, an occasional small lymphocyte contained radioactivity. Mature neutrophils and eosinophils were not observed to label.

Although biochemical analyses left no doubt that only RNA was administered, Figs. 1*B* and 1*D* demonstrate that occasionally radioactivity was found in both normal and neoplastic cells after ribonuclease digestion. That this radioactivity represented incorporation of labeled materials into DNA is suggested from the discrete nuclear localization of the label. Furthermore, the complete disappearance of nucleolar and cytoplasmic radioactivity caused by ribonuclease treatment strongly argued against the label remaining in nuclear RNA because of enzyme failure. In other experiments label of this type was specifically removed by deoxyribonuclease digestion. It was concluded that both DNA and RNA were labeled by the injected RNA, but that much the larger part of the label appeared in RNA.

Whether the macromolecular RNA was partially degraded before being incorporated could not be definitively determined. That a polymerized nucleic acid (DNA) may be incorporated intact in HeLa cells has been shown by Borenfreund and Bendich (*8*). In the present work occasional clumps of cytoplasmic radioactivity suggesting pinocytotic vesicles of RNA were observed at early intervals after administration of RNA. Whether the label observed in nuclei and nucleoli at the same intervals derived from the cytoplasmic clumps or from direct incorporation of RNA catabolites was uncertain.

Cohen (*21*) has postulated that RNA may serve as a precursor for DNA at the nucleotide level. The present finding of label in DNA of occasional recipient cells may constitute support for this concept, as well as suggest some degradation of the RNA either before or after incorporation. The fact that by far the larger part of the label was found in the RNA of recipient cells rather than distributed relatively equally between RNA and DNA argues against complete breakdown of the administered RNA to a precursor common to both nucleic acids. In summary, it appears that administered radioactive RNA is most effective in labeling RNA of recipient cells, but also labels DNA to a slight extent. The molecular species effecting this labeling are unknown but those that label DNA probably represent small degradation products (*22*).

M. Roy Schwarz*
William O. Rieke
Department of Anatomy,
University of Washington
School of Medicine, Seattle 5

References and Notes

1. M. Hess, J. J. Corrigan, Jr., J. A. Hodak, *Endocrinology* **68**, 549 (1961).
2. M. C. Niu, *Proc. Natl. Acad. Sci. U.S.* **44**, 1264 (1958); J. Brachet, *The Biological Role of Ribonucleic Acids* (Elsevier, New York, 1960); ———, *Embryologie chimique* (Desoer, Liége, 1944); T. Kuusi, *Arch. biol. (Liége)* **64**, 189 (1953); T. Yamada, *Experientia* **14**, 81 (1958).
3. F. L. Ashley, E. G. McNall, N. R. Dutt, E. N. Barcia, R. F. Sloan, *Ann. N.Y. Acad. Sci.* **87**, 429 (1960); M. Hrubesova, B. A. Askonas, J. H. Humphrey, *Nature* **183**, 97 (1959).
4. B. Panjevac, G. Ristic, D. Kanazin, in *2nd U.N. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1958* **23**, 64 (1958).
5. S. DeCarvalho and H. J. Rand, *Nature* **189**, 815 (1961).
6. M. C. Niu, C. C. Cordova, L. C. Niu, *Proc. Natl. Acad. Sci. U.S.* **47**, 1689 (1961).

7. S. M. Gartler, *Nature* **184**, 1505 (1959); K. Bensch and D. W. King, *Science* **133**, 382 (1960); M. Hill and V. Drásil, *Exptl. Cell Research* **21**, 569 (1960).
8. E. Borenfreund and A. Bendich, *J. Biophys. Biochem. Cytol.* **9**, 81 (1961).
9. E. Kay, *Nature* **191**, 387 (1961).
10. A. Ficq, *J. Embryol. Exptl. Morphol.* **2**, 194 (1954).
11. H. Amos, *Biochem. Biophys. Research Comm.* **5**, 1 (1961).
12. K. S. Kirby, *Biochem. J.* **64**, 405 (1956).
13. H^3-cytidine, New England Nuclear Corporation, Boston, specific activity 8.1 count/mmole.
14. Deoxyribonuclease, Worthington, Freehold, N.J., 1 mg/ml, phosphate buffer *p*H 7.0, 37°C, 0.01*M* Mg^{++}.
15. G. Ceriotti, *J. Biol. Chem.* **198**, 297 (1952).
16. W. Mejbaum, *Z. physiol. Chem. Hoppe-Seyler's* **258**, 117 (1939).
17. H^3-uridine, Schwarz, Mt. Vernon, N.Y., specific activity 0.38 count/mmole.
18. H^3-adenine, New England Nuclear Corporation, Boston, specific activity 1.35 count/mmole.
19. N. B. Everett, W. O. Rieke, W. O. Reinhardt, J. M. Yoffey, in *Ciba Symposium on Hemopoiesis* (Churchill, London, 1960), p. 43.
20. Ribonuclease, Mann Research Lab., New York, 0.5 mg/ml phosphate buffer *p*H 6.7, 37°C.
21. S. S. Cohen, *Cancer Research* **20**, 698 (1960).

22. This work was supported in part by PHS grant RG-6309 and by graduate research training grant CRT 5040 from the National Cancer Institute.

* Graduate research trainee CRT.

5 January 1962

Direct Tabular Method for Obtaining the Order of a Reaction of a Restricted Class

Abstract. When the rate law of a process can be written in the form $X' = dx/dt = -kX^m$, the order $m = XX''/(X')^2$ may be calculated directly, in lieu of the usual trial-and-error methods. An analytical method is used to calculate the derivatives X' and X'' from the collected data, X, and t.

Commonly, a trial-and-error procedure is employed for finding the order of a reaction (*1*). It should be possible to obtain the order directly (*2*) for reactions of that restricted class whose rate equation may be written in the form

$$x' = -kx^m \qquad (1)$$

Upon differentiating with respect to time, and rearranging, this becomes

$$m = x'' x/(x')^2 \qquad (2)$$

Data are usually collected as concentrations and so forth (that is, x), and the two differentiations can be performed by the tabular method (*3*, p. 455). Where the rates of change (that is, x') are the collected data, tabular methods of integration are available (*3*, p. 456; *4*, p. 94) for calculating x values.

In the example shown here the datum is the mass, M grams, of unreacted vitreous silica, as a function of time, in the presence of a stoichiometrically equivalent amount of hydrofluoric acid. The rate of solution in this experiment depends on the surface area, S square centimeters, of silica powder, and on the hydrofluoric acid concentration, L/V moles per kilogram of water. Thus,

$$dM/dt = -kS\ (L/V)^n \qquad (3)$$

where n is the partial order of the reaction with respect to the hydrofluoric acid concentration. From the overall reaction (*5*)

$$6\ HF + SiO_2 \rightarrow H_2SiF_6 + 2H_2O$$

and the fact that the two reactants are present initially and during the course of the reaction, in stoichiometrically equivalent amounts (at $t = 0$, $M_0 = 2.0180$ g, $L_0 = 0.2016$ mole of hydrofluoric acid, $V = 0.200$ kg of water; the solution is also 1 molal in hydrochloric acid),

$$L \text{ mole HF} = 0.09990\ M \text{ g } SiO_2 \qquad (4)$$

The assumption is made that the surface S varies as the ⅔ power of the mass of remaining silica (*6*),

$$S = S_0\ (M/M_0)^{\frac{2}{3}} \qquad (5)$$

where $S_0 = 680$ cm²/g.

By substitution of Eqs. 4 and 5 into Eq. 3, this last becomes

$$dM/dt = -k\ (S_0/M_0^{\frac{2}{3}})\ (0.4995)^n\ M^{n+\frac{2}{3}} \qquad (6)$$

which is in the same form as Eq. 1.

The equations for differentiation (*2*) are

$$(dM/dt)_i = \frac{1}{h}\left[\Delta M_i - \frac{1}{2}\Delta^2 M_i + \frac{1}{3}\Delta^3 M_i - \ldots\right] \qquad (7a)$$

$$(d^2M/dt^2)_i = \frac{1}{h^2}\left[\Delta^2 M_i - \Delta^3 M_i + \ldots\right] \qquad (7b)$$

where h is the common interval of the time variable (in this case, 1800 sec). Table 1 lists the calculations needed for obtaining the order and the rate constant. Since second and third order differences are used, precise data which are either accurate or amenable to smoothing techniques (*4*, p. 6) are required. Column 3 in the table is the data after one smoothing process. Not all smoothing processes lead to the same results. Two other attempts gave, for the partial order, $n = 0.93$, and $n = 1.11$, and, for the rate constant, $k = 7.12 \times 10^{-8}$ and 7.16×10^{-8} g of SiO_2 per square centimeter, per second, per HF molality. The latter were calculated from Eq. 6, where $n = 1$. These results agree with those obtained by other methods (*6*).

From Eq. 1 or Eq. 6 it is apparent that plotting log $(-M')$ versus log M should give a straight line whose slope

Table 1. Table for obtaining the order of a reaction of a restricted class (T = 32.1 °C).

t (sec)	M_{exp}(g)	$M_{smoothed}$	ΔM	$\Delta^2 M$	$\Delta^3 M$	M'	M''	$n + \frac{2}{3}$	$k\left(\frac{\text{g } SiO_2}{\text{sec cm}^2 \text{ HF molality}}\right)$
0	2.0180	1.9916							
1800	1.9124	1.9129	−0.0787						
3600	1.8265	1.8393	−0.0736	+0.0051					
5400	1.7742	1.7704	−0.0689	0.0047	−0.0004	-3.94×10^{-5}	1.57×10^{-9}	1.79	7.14×10^{-8}
7200	1.6931	1.7056	−0.0648	0.0041	−0.0006	-3.72×10^{-5}	1.45×10^{-9}	1.79	7.18×10^{-8}
9000	1.6378	1.6447	−0.0609	0.0039	−0.0002	-3.50×10^{-5}	1.26×10^{-9}	1.69	7.18×10^{-8}
10800	1.5904	1.5874	−0.0573	0.0036	−0.0003	-3.29×10^{-5}	1.20×10^{-9}	1.76	7.17×10^{-8}
12600	1.5467	1.5332	−0.0542	0.0031	−0.0005	-3.11×10^{-5}	1.11×10^{-9}	1.76	7.18×10^{-8}
14400	1.4911	1.4821	−0.0511	0.0031	.0000	-2.93×10^{-5}	0.96×10^{-9}	1.66	7.15×10^{-8}
16200	1.4462	1.4338	−0.0483	0.0028	−0.0003	-2.76×10^{-5}	0.96×10^{-9}	1.81	7.13×10^{-8}
18000	1.3699	1.3881	−0.0457	0.0026	−0.0002	-2.62×10^{-5}	0.86×10^{-9}	1.74	7.13×10^{-8}
								1.75 ± .04	$7.16 \pm .02 \times 10^{-8}$
								$n = 1.08 \pm .04$	