

vitamin content. It seems probable that smaller quantities of mud could be dried rapidly at 100°C without loss in vitamin-B₁₂ activity (7).

Our attempts to perform direct assays on dissolved vitamin B₁₂ in sea water have not been successful thus far, even with *E. coli* that was especially adapted to high salinity. This aspect of the problem is being examined with studies on other bacteria that may prove more suitable for the task.

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Suppression and Modification of Virus-Induced Rous Sarcoma in Chicks by Xerosin

This report (1) presents data showing that parenteral injections of xerosin both suppressed and modified virus-induced Rous sarcoma in chicks and that the effectiveness of xerosin was markedly influenced by the initiating dose of Rous sarcoma virus (RSV). This observation is of particular interest because it has been previously shown (2) that the amount of RSV extractable from a given tumor was directly related to the amount of RSV used to initiate that tumor. Indeed, when high dilutions of RSV were employed which produced tumors in less than 50 percent of the inoculated chicks, about 24 percent of such low-dose tumors yielded no extractable virus at all. Further, the latent period of tumor response (2-4) and the rate of growth of the tumor (2) were also found to be related to the initiating dose of RSV (3).

Stable, standard, frozen inocula of RSV (5) were used. The bacterial product, xerosin, was prepared as previously described (6). Suitably diluted RSV in 0.2-ml amounts was inoculated subcutaneously into the wing web of unsexed white leghorn chicks 2 to 5 days of age. Single daily injections of 100 mg/kg of xerosin were injected intramuscularly in the leg. Each chick was examined daily.

The data were analyzed graphically as previously illustrated (4, 7) for data of this type.

Table 1 shows the effect of the initiating dose of RSV on the effectiveness of xerosin in modifying tumor response. Chicks were injected daily with xerosin or saline, respectively, beginning 2 days before inoculation of RSV. The data show that, when chicks were inoculated with dilute RSV (for example, 10⁻⁶), xerosin prolonged the latent period more effectively, and the incidence of atypical tumors was markedly greater. The most striking effect of xerosin on Rous sarcoma was the greatly increased incidence of atypical tumors. Typical tumors were soft, grew rapidly, and were grossly invasive, while the xerosin-induced atypical tumors were hard, sharply circumscribed, and grew slowly. A representative typical and atypical tumor are pictorially presented in Fig. 1.

Daily injections of xerosin were discontinued 3 weeks after inoculation of RSV in 10 chicks with atypical tumors, and these chicks were examined daily for 2 additional weeks. These atypical tumors continued to grow slowly, but none reverted to a typical grossly invasive tumor. Next, each of these atypical tumors was removed and each was mascerated with sand in a mortar, suspended in 9 volumes of saline, and clarified by centrifugation, and each suspension was inoculated subcutaneously into groups of 10 chicks each. Three weeks later, the results clearly indicated that each atypical tumor contained virus that produced typical invasive tumors after subinoculation.

Modification of the Rous sarcoma was also effected when daily injections of xerosin were delayed until the day on which typical small but grossly visible tumors appeared in the wing web. In this

Table 1. Effect of initiating dose of RSV* on modification of tumor-response in chicks by xerosin.

Standard RSV diluted (log)	Daily intra-muscular injection of	Tu/T†	Mean latent period‡ (days)	Atypical tumors (%)
-6	Saline	31/39	7.0	8§
-6	Xerosin	18/31	11.3	52
-5	Saline	39/40	6.7	3
-5	Xerosin	30/32	7.9	8
-4	Saline	40/40	6.1	0
-4	Xerosin	25/27	6.7	0

* RSV—Rous sarcoma virus (chicken tumor I agent).

† Tu/T = Number of chicks with tumors/total.

‡ Mean latent period = time in days to produce grossly visible tumors in 50 percent of chicks.

§ Slow-growing, circumscribed tumors may also occur in untreated chicks inoculated with small doses of RSV.

|| 100 mg/kg day beginning 2 days before RSV.

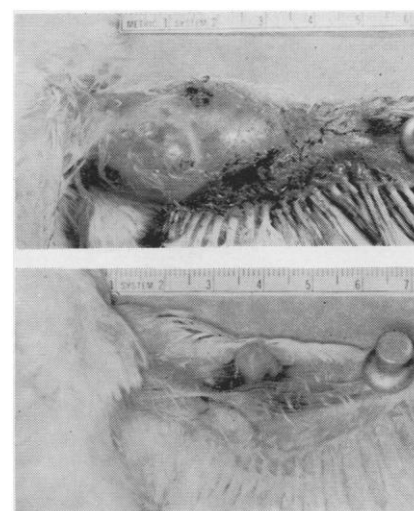


Fig. 1. Typical and xerosin-induced atypical tumors 19 days after inoculation of approximately 10 ED₅₀ of RSV. (Top) typical; (bottom) atypical.

experiment, 300 chicks were inoculated in the wing web with RSV diluted 10⁻⁶. One-half of the chicks were set aside for injections of xerosin and the other half for saline. Daily intramuscular injections of xerosin or saline were begun for each chick, individually, on the day when a tumor 3 mm or more in diameter appeared in the translucent wing web. Four weeks after inoculation of RSV, 98 of 150 chicks in the control group had well-established tumors, 15 percent of which were atypical, while 101 of 149 chicks in the xerosin-treated group had well-established tumors, 57 percent of which were atypical. It is clear that the incidence of xerosin-induced atypical tumors was almost identical whether daily injections of xerosin were begun before inoculation of low doses of RSV or delayed until the tumors first appeared in the wing web.

There is a striking similarity between the gross appearance of the atypical tumors induced by xerosin (Fig. 1) and those induced by hydrocortisone (8). However, the latter promptly reverted to typical invasive tumors when hydrocortisone was discontinued, and, when injections of hydrocortisone were begun after inoculation of RSV, the tumors were not only typical but also grew more rapidly than control tumors. Ginsberg (9) has shown that, while both xerosin and cortisone beneficially modify pneumonia that has been induced in mice by chemical irritants, only xerosin beneficially modifies pneumonia in mice that are infected with mouse-unadapted influenza virus. Despite the fact that xerosin lacks any antiviral or other antimicrobial activity whatever (6, 9), and therefore cannot be classified as an antibiotic, xerosin was found to beneficially modify disease induced in mice by the

pneumotoxicity of Newcastle disease virus (10) and by the neurotoxicity of influenza virus (11).

To the extent that the Rous sarcoma is typical of other virus-induced tumors, it is important that tumor response to at least one chemotherapeutic agent is greatly influenced by the amount of virus used to initiate the tumor.

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References and Notes

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On the Intimate Composition of Membranes of the Inner Ear

In 1953 (1), autoradiographic studies of radiosulfate incorporation by the inner ear were conducted in our laboratory. This work revealed a relatively high uptake by the membrana tectoria and also by the gelatinous mass of the cupula, while the otolithic membrane of the macula recorded a lower concentration. Radiosulfate ($S^{35}O_4$) was also detected in all three membranes by Ringertz (2), who used a similar approach. Furthermore, our ethanol-formalin fixed, undecalcified tissues stained metachromatically with toluidine blue in all the areas that have revealed an uptake of radiosulfate (3) including the inner-ear membranes (1). These results seemed to indicate that the radiosulfate was retained as newly synthesized sulfomucopolysaccharides.

Recently, Wislocki and Ladman (4) have challenged this hypothesis on the basis of studies of decalcified newborn-mouse tissue fixed in lead acetate or Zenker's acetic acid fluid which revealed no metachromasia. On the other hand, those authors have obtained a positive

result with the Barnett and Seligman test for SH_2 and $S-S$ groups and conclude that the uptake of S^{35} which we had observed might be explained by the formation of disulfide groups associated with cystine.

If there was a concentration of sulfur-containing amino acids in the inner ear membranes, such as is encountered in keratinized tissues, S^{35} -labeled cystine or methionine (5) would be expected to produce a more intense autoradiographic reaction in these sites than $S^{35}O_4$, for the amount of cystine and methionine biosynthesized from sulfate in mammalian tissue is known to be very small (6).

While currently conducting a general survey of uptake of S^{35} -labeled methionine and cystine (7), we have had the opportunity to observe several specimens of the inner ear in rats labeled at 8 days of age and sacrificed at intervals of 1, 2, and 6 hours and 1, 2, and 4 days thereafter (8). The histoautoradiographic processing has been the same as previously reported for the radiosulfate series (1, 3). The tissues that are known to contain keratin—the epidermis, hair, and tooth enamel—revealed a high uptake of labeled amino acids.

Although cystine is the most important sulfur-containing amino acid of the keratins, it has been shown by Tarver and Schmidt (9) that when labeled methionine is introduced into an experimental animal, a large proportion of the radioactive sulfur appears in crystallized cystine from hair and skin. This is an indication that there is conversion from methionine to cystine at the level of the tissues. The other structures, synthesizing proteins with a low concentration of sulfur-containing amino acids, showed a much lower graded uptake, which was assumed to be proportional to the local rate of synthesis (7).

The inner ear, by comparison with other regions of nonspecific uptake such as the bone (Fig. 1), is definitely an area of low concentration, producing an autoradiographic record only after exposures of several months. Within this structure, certain features such as the area vasculosa and the spiral lamina appear to be more active than the general tissue background. On the other hand, the tectorial membrane (Fig. 1, arrows), the cupula, and the otolithic membrane were constantly negative.

These results must be compared with the large concentration of $S^{35}O_4$ previously reported (1) and presumably bound to large polysaccharide molecules. Furthermore, it has been possible to reproduce on the present material, with or without demineralization, the strong periodic acid-Schiff staining of the membranes that was described by Wislocki and Ladman (4).



Fig. 1. Integrated autoradiogram of the cochlea of a 9-day-old rat, 24 hr after an injection of S^{35} -methionine ($\times 29$). The organ of Corti shows a general low uptake. The tectorial membrane (arrows) is negative.

It seems evident that the inner-ear membranes contain a large amount of polysaccharides and that these are at least partly sulfated. On the other hand, as compared with the skin, hair, and enamel, the inner-ear membranes synthesize virtually no protein from S^{35} -methionine.

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Effect of Sensory Deprivation on Learning Rate in Human Beings

Recent theoretical considerations (1) concerning brain functioning have so emphasized the necessity for constant sensory bombardment in order to maintain normal, intelligent, adaptive behavior that it is only natural to speculate about the effects of reduced sensory stimulation. As interesting as it might prove to be, it is obviously not possible to stop completely all sensory input and still maintain a responsible observer. It is possible, however, by rigid conditions of confinement, to minimize not only the amount of sensory input but also to bring about a drastic reduction in its variability. Such a condition of confinement, to be