Direct instillation of "citrovorum factor" into the bone marrow cavity was performed in 3 persons with pernicious anemia in relapse; the amounts used were 0.06 mg, 1.5 mg, and 3 mg, respectively. In no instance was there evidence on Wright-Giemsa–stained marrow smears of the erythrocyte maturation effect that was observed locally after marrow instillation of vitamin B_{12} (*8*) but which did not occur after the instillation of 1 or 2 mg of folic acid into the marrow. In this respect, also, folinic acid is similar to folic acid.

This study demonstrates that "folinic acid" or "citrovorum factor" is a potent hematopoietic agent in pernicious anemia in relapse, but is no more effective than a similar dose of folic acid. The failure of the substance to produce a local erythrocyte maturation effect on instillation into the marrow cavity suggests that "citrovorum factor" or "folinic acid," like folic acid (*10*), must be altered elsewhere in the body before becoming active in hematopoiesis.

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Otitis Media and Audiogenic Seizures in Mice[1]

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Infection of the middle ear in rats has been shown to be a factor in the occurrence of audiogenic seizures (*1–3*). The original report by Patton (*2*) stressing the complications that might thus arise in using the incidence of seizures in rats as an index of nutritional deficiency has, however, been misinterpreted by some workers. They seem to believe that Patton proved that purulent otitis media is the only and sufficient cause of audiogenic seizures. Patton, however, stated that his observations "do not define the role of middle ear disease in the etiology of sound induced seizures," and that "the infection has not complicated the severe sound induced seizures associated with specific deficiencies, e.g., magnesium. . . ." Sound-induced seizures in rats are thus possible without concomitant otitis media (*1–3*). As Pilgrim and Patton (*3*) state, "The precise relationships between convulsions and the infection have not yet been elucidated."

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Laboratory mice exhibit audiogenic seizures similar to those of rats, and mice may be better than rats as test animals for certain purposes (*4*). Before mice are widely used for tests of auditory reactions, however, it seems advisable to determine the relationship, if any, between otitis media and seizures in these animals.

Mice of three strains were used in this study: dba Subline 1, C-57 black, Subline 6, and mongrel albino, so-called Swiss. The first two were obtained originally from the market stock of The Jackson Memorial Laboratory, Bar Harbor, Maine, the third from an animal dealer. All individuals used in our experiments were reared in our laboratory.

Seizures were induced by subjecting mice imprisoned in a small wire-mesh cage to a sound field at 10 kc frequency and 110 db average sound pressure. The animals were tested daily from 15 to 50 days of age. Mice of all three strains are susceptible during some part of this period. Details of the apparatus and procedures have been published elsewhere (*5*). The occurrence of otitis media was determined by autopsy carried out under a binocular dissecting microscope. The bulla and tympanum were exposed and penetrated, and the middle ear was carefully examined for inflammation and pus.

The mice autopsied were of the following classes: (1) animals which either had no seizures during the test period or had seizures during the early part of the period (20–30 days of age) but stopped having seizures at least 10 days before the examination; and (2) animals which died as a result of clonic-tonic seizures. The second group certainly includes the most susceptible mice in the colony. In the first group (controls) were 70 albinos, 10 dba's, and 10 C-57's; in the second group were 131 albinos, 53 dba's, and 16 C-57's. Approximately half the mice in each group were males and half females, and they varied in age from 18 to 50 days, most of the animals that died being 20–30 days old.

No case of otitis media was found in the control group, and only one case of the disease was found in animals dying in seizure, a unilateral infection in a dba. One other case appeared during the study. An albino which had only 2 seizures, at 20 and 21 days of age, developed, at 41 days of age, definite symptoms of middle ear infection and labyrinthitis, holding the head to the side and swinging in a circle when held by the tail. Dissection of the middle ear, when the mouse was 42 days old, confirmed the diagnosis. Since this animal had a low seizure record, it seemed advisable to examine animals with similar records but without clinical symptoms of the disease. Twenty albino mice with similar records were examined, and no case of otitis media was found.

It is obvious that the incidence of otitis media in our colony of laboratory mice is very low. This accords well with the report of Caussé (*6*) that otitis media is found in at most 1% of white mice. The low incidence found here is matched by that discovered quite independently for dba's and C-57's by Miller and

Zamis (*7*). These results contrast strikingly with reports by the workers with rats (*1, 2*) that 80–95% of their animals were infected.

So far as mice are concerned, therefore, otitis media is not necessary for susceptibility to audiogenic seizures. Further, the very low incidence of the disease in mouse colonies makes it negligible as a complication in studies on auditory reactions.

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Immunochemical Changes in Chicken Serum During Development of Rous Sarcoma[1]

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It is well known (*1–9*) that during the growth and maturation of the individual changes occur in the serum, manifested by an increase in globulin and by the appearance of various antibodies for cells of foreign species and multiple infectious agents. In the chicken one finds antibodies against tumor viruses, e.g., the Rous sarcoma (*3*), and also, as recently found in this laboratory, against *Proteus*, a bacterium that thrives in autolyzed extracts of this same sarcoma.

Furthermore, it is recognized (*1*) that, as a common denominator to the development of natural or experimentally induced antibodies in chickens, a factor appears in the globulin fraction of the serum which is endowed with the property of flocculating, in the cold, saline or alcoholic extracts of many tissues from many species of animals.

On the other hand, it has been found by several workers (*4–6*) that chickens bearing the Rous sarcoma or a lymphocytoma show a hypoproteinemia depending not on changes in the plasma volume (*6*) but rather, in the case of the lymphocytoma, on a reduction of albumin and occasionally of globulin.

In our studies on the immunochemical changes in the serum during malignancy, we focused our attention on the naturally present antibodies as well as on the factor flocculating tissue extracts, and it was soon apparent that in chickens bearing the Rous sarcoma there was a diminution in some of these immune bodies. Although this decrease was quite evident with the globulin factor flocculating tissue extracts in our test (mouse liver), it was most clear-cut in an agglutination reaction with *Proteus*. We paralleled these studies with an analysis of serum protein and have compared the results of both methods in the following note.[3]

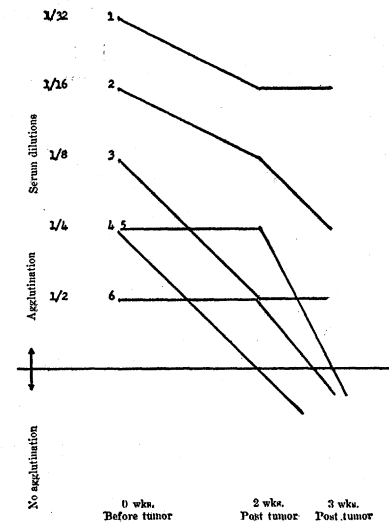

FIG. 1. Decline of *Proteus agglutinin* in chicken serum during development of Rous sarcoma.

Proteus agglutinin can be readily detected by combining serum with a live culture, vaccine, or O antigen of the *Proteus vulgaris* or *OX19* strains, both of which are equally suitable.[4] The organisms were grown in broth for 24 hr and then transplanted to agar for the same length of time. The agar growth was suspended in physiological saline, to which we added 30% by volume of alcohol and incubated the mixture overnight at 37° C. At this stage the bacilli were usually dead and could be centrifuged to obtain a precipitate which was resuspended in 7 equal volumes of saline. Before testing, this initial stock solution was further diluted 10–15 times with saline, depending upon trial tests necessary to determine the greatest dilution that would give agglutination with normal central sera. The actual test was done in small test tubes (1.2 cm × 10.1 cm) protected with cork stoppers. To each tube containing 0.1 ml of serum, inactivated or not, undiluted or in dilution, we added 0.1 ml of antigen and, after incubating at room temperature, read the tubes with the aid of a binocular microscope (10 × and 23 ×) at several time intervals over a 2-hr period. Similarly, the presence of the tissue flocculating factor in chicken serum was indicated by flocculation with a 5% saline extract of mouse liver kept at 0° C for 24 hr.

Both determinations indicated a high incidence of

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[2] National Cancer Institute special research fellow.

[3] While this paper was in press, the Lankenau Hospital reported similar results with mice (*12*).

[4] The strains were obtained from the American Type Culture Collection. In the earliest stages of our work, however, we used *Proteus* which was isolated and identified through the kindness of Leanor Haley, from a contaminated extract of chicken sarcoma.