doubt that any very remarkable effects would be forthcoming from the use of dilute solutions.

In this connection, the writer felt that it might be interesting to determine the effect of a dilute solution of deuterium oxide upon the growth of a fungus, *Aspergillus sp.* This organism has been found to be extraordinarily sensitive to differences in the culture medium (Mann[4]) and so might be expected to reflect in its growth and fruiting any changes brought about by the presence of the isotope of hydrogen.

A sample of deuterium oxide containing one part deuterium to 213 parts protium, approximately a .47 molecular per cent. solution, was obtained from the Ohio Chemical and Manufacturing Company at Cleveland. This water was distilled in order to remove any impurities present. After distillation, the relative density of the heavy water referred to ordinary water, both at 20° C., was 1.0019.

The nutrient solution used in these experiments was Pfeffer's three-salt solution, with sucrose as the source of carbon. Two groups of nutrient solutions were prepared, one with double-distilled H^1H^1O; the other with distilled H^2H^2O. The nutrient medium was placed in 150 cc Erlenmeyer flasks, 50 cc to the flask. The solutions were sterilized by streaming steam for a period of twenty minutes on each of three successive days. Inoculations were made from a pure, bacteria-free culture of *Aspergillus sp.* on bread by means of a platinum loop. The fungus was grown in an incubator for five days at a temperature of 37° C. The mycelial felts were then removed from the flasks, placed on weighed filter paper and dried for three days at 65° C. The filter paper used had been previously dried at the same temperature and placed in a desiccator. At the end of the three-day period, the felts were removed from the drying oven, placed in a desiccator and then weighed. From the total weight of the felt and the filter paper was subtracted the weight of the filter paper alone. Thus it was possible to determine and compare the weights of the felts grown in the deuterium oxide medium with those grown in that of protium oxide.

The fungus grown in the H^1H^1O Pfeffer's medium was in the form of a flat and evenly fruited felt. The average weight of the felts from four flasks of this solution was .0481 grams. The felts grown in the heavy water medium exhibited every indication of stimulation. They were markedly convoluted and cratered below, resembling a brain-coral; the fruiting was greatly diminished, and the distribution of spores on the surface of the felts was irregular and occurred in localized regions. When the dry weights of these heavy water felts were taken, it was found that the average weight from the four flasks was .7719 (see Table I), or approximately sixteen times that of the felts grown in the ordinary distilled water medium.

TABLE I*

Series	Weight of filter paper	Weight of filter paper and felt	Weight of felt
1 a	1.3544 gr.	1.3963 gr.	.0419 gr.
1 b	1.3490 gr.	1.3994 gr.	.0504 gr.
1 c	1.3511 gr.	1.3983 gr.	.0472 gr.
1 d	1.3593 gr.	1.4123 gr.	.0530 gr.
Average weight of felt = .0481 grams.			
2 a	1.3592 gr.	2.1400 gr.	.7808 gr.
2 b	1.3722 gr.	2.1940 gr.	.8218 gr.
2 c	1.3681 gr.	2.0724 gr.	.7043 gr.
2 d	1.3442 gr.	2.1250 gr.	.7808 gr.
Average weight of felt = .7719 grams.			

*Series 1—Pfeffer's Three-Salt Solution + Distilled H^1H^1O.
Series 2—Pfeffer's Three-Salt Solution + Distilled H^2H^2O.

The writer, therefore, is of the same opinion as Barnes and Richards, that deuterium, when used in dilute concentrations, may have a decided effect in stimulating vegetative growth and development. Further experiments in this connection are planned and a more detailed consideration of the methods used and the results obtained will be published later.

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THE EFFECT OF FERRIC CHLORIDE INJECTIONS IN EXPERIMENTAL TUBERCULOSIS[1]

SEVERAL years ago the writer showed that repeated intravenous injections of a 0.25 per cent. ferric chloride solution were followed by an accumulation of iron in caseous areas of the lungs of tuberculous rabbits.[2,3] Concomitantly with this accumulation of iron, the life span of infected animals was found considerably increased over that of control tuberculous rabbits that had received no injections of the ferric salt solution. This was found to be the case in two entirely independent series of experiments comprising 36 rabbits.[4,5] Furthermore by comparing tuberculous lesions of both experimental and control animals sacrificed at various intervals of time, it was shown that following the intravenous injections of ferric

[4] M. L. Mann, "Calcium and Magnesium Requirements of *Aspergillus niger*," *Bull. Torrey Club*, 59: 443–490, 1932.

[1] From the Department of Pathology, Harvard Medical School, Boston, Mass. Aided by a grant from the DeLamar Mobile Research Fund.

[2] V. Menkin, *Proc. Soc. Exp. Biol. and Med.*, 27: 1020, 1930.

[3] V. Menkin and M. F. Menkin, *Jour. Exp. Med.*, 53: 919, 1931.

[4] V. Menkin, *Jour. Exp. Med.*, 55: 101, 1932.

[5] V. Menkin, *Am. Jour. Med. Sci.*, 185: 40, 1933.

chloride, the progress of the lesions was retarded in their extent.[5] The conclusion drawn was that ferric chloride protracted the course of bovine tuberculosis in the rabbit. Subsequent studies revealed the fact that subcutaneous inoculation of tubercle bacilli followed by an immediate course of intravenous injections with dilute ferric chloride solution retarded the dissemination of the organisms, as evidenced by the degree of tuberculous involvement in the various organs.[6]

These studies have been continued and form the subject of this brief communication. A more detailed account of the following observations will appear in a subsequent report. Ten rabbits were each inoculated intravenously on November 8, 1932, with 0.005 mgm of a relatively avirulent strain of bovine tubercle bacilli (Strain Cernay, obtained through the kindness of Dr. A. Boquet from the Pasteur Institute). On December 22, 1932, each one of these 10 rabbits was inoculated subcutaneously in the thigh with 0.05 mgm of a virulent bovine Ravenel strain of tubercle bacilli. Repeated intravenous injections with 0.25 per cent. ferric chloride solution were immediately started in 5 of the rabbits; the remaining tuberculous animals were kept as controls. The ferric chloride injections were carried on for about 15 weeks and then discontinued. The first control rabbit died 47 days after reinfection with tubercle bacilli. On May 1, 1933, 130 days after reinoculation with the bacilli, the last of the controls was found dead. Practically all these 5 control animals displayed at autopsy wide-spread confluent tuberculous lesions, affecting primarily the lungs, with discrete tubercles in the kidneys, and with prominent tuberculous abscesses at the site of subcutaneous reinoculation. The average survival time of the control group was 81.4 days.

The first experimental rabbit died 81 days after reinoculation; the second died on the 110th day; and the third one succumbed 131 days after reinfection with tubercle bacilli. Two of these three animals had an upper respiratory infection at the time of death. All three rabbits revealed discrete caseous tubercles in the lungs, with hardly any confluence of lesions. The two remaining animals continued to increase in weight for over 6 months after the death of the last control. On November 21, 1933, 334 days after reinoculation with tubercle bacilli, these two rabbits were killed, and Professor S. B. Wolbach very kindly performed the post-mortem examinations. One of these two animals showed absolutely no sign of any lesion in the thigh. This fact, though somewhat surprising, is not wholly incomprehensible when it is recalled that Lurie recently failed to find in some rabbits any trace of local lesions about one year following subcutaneous inoculation with human tubercle bacilli, whereas in other animals he found large well-walled-off tuberculous abscesses.[7] Near the base of the lungs two large tuberculous foci were found, one of which formed a pus pocket in which tubercle bacilli swarmed in abundance. These lesions were well walled-off by connective tissue proliferation. The other rabbit, which had just begun to show slight loss in weight at the time that it was killed (334 days after reinoculation with tubercle bacilli), revealed at autopsy somewhat more tuberculous involvement. In the thigh at the original site of subcutaneous inoculation there was found a prominent well-encapsulated tuberculous abscess containing a thick viscous creamy purulent exudate, in which were found numerous tubercle bacilli. Throughout the lungs were scattered a number of discrete tuberculous foci with no confluence of lesions. On microscopic examination the areas of caseation in these tubercles were found to be relatively small in comparison with the peripheral cellular infiltration. The extent of tuberculosis was considerably less than in the controls, the last of which had died more than 200 days previously. The spleen of this experimental animal revealed a single large caseous focus. The left kidney showed considerable caseous involvement of the cortex and medulla with the process extending to the pelvis and the proximal portion of the left ureter. A few discrete tubercles were found in the intestinal tract primarily in the region of the cecum.

The average survival time of the experimental group is unknown, since two of the animals were finally sacrificed. However prior to the autopsy of the two experimental survivors the average life span of this group of rabbits was 198 days, as compared with 81.4 days in the control series. These observations are not only in complete agreement with the findings obtained in the previous and independent series of experiments,[4, 5, 6] but they reveal even more pronounced effects upon the course of tuberculosis in rabbits repeatedly treated with ferric chloride. In none of the previous experiments were there any tuberculous animals which survived in apparent good health, with consistent gain in weight for a period of over 6 months after the death of the last of the controls. It remains to be seen whether this sharper retardation in the development of tuberculosis is entirely due to the ferric chloride injections or whether it may be due in part to the combined effects of a superimposed initial vaccination. Studies now under way in a series of non-reinfected tuberculous rabbits seem to show that primary infection with an avirulent strain of bacilli probably plays only a rela-

[6] V. Menkin, *Proc. Soc. Exp. Biol. and Med.*, 30: 951, 1933.

[7] M. B. Lurie, *Jour. Exp. Med.*, 58: 305, 1933.

tively minor rôle in delaying the progress of the disease induced by reinfection with a virulent strain. The more striking results in regard to longevity and progress of tuberculosis obtained in the present series of experiments as compared to previous series are perhaps referable to various other factors, including route of virulent tubercle bacillus inoculation, *i.e.*, subcutaneous instead of intravenous, interval of time between inoculation and onset of intravenous ferric chloride injections, and finally, dose and virulence of bacilli employed. Experiments are now in progress in an attempt to answer these various questions. They will form the subject of a future communication.

VALY MENKIN

FROGS AND OPALINIDAE

CONCOMITANT studies of the taxonomy and geographical distribution of the Anura and their intestinal commensals, the Opalinidae, give indications of places and geologic times of origin and of the routes and times of spreading of all but one of the families of Anura, of some subfamilies and of several genera: and at the same time tell of the times and places of origin and of the routes and times of spreading of the Opalinidae, about 130 species of these Protozoa being included in the survey. A study of these problems has been in the hands of the editor for the United States National Museum since May, 1932, waiting only for the restoration of customary appropriation for printing. Such detailed study of origin and distribution has not been given before for any groups of animals. The data used are interwoven in intricate ways and are difficult to discuss without full description, but because of the unavoidable delay in publication it seems advisable to attempt the presentation of enough to show the kinds of data used, the methods of using them to bring out their significance and to illustrate the extent of the conclusions reached.

The data are: Present geographical distribution of the species and groups of Anura and of the species and groups of Opalinidae; the host distribution of Opalinidae; the taxonomic relationships of the groups of Anura; the interrelationships in detail of the Opalinidae, which show from their anatomy and their life-histories the course of their evolution in great detail; the agreement of present-day distribution of hosts and parasites with ideas of former intercontinental land connections and also seas, as commonly postulated by students of paleocartography.

It is of interest that the almost complete absence of paleontologic record of the Anura does not prove an insuperable bar to such evolutionary and paleodistributional studies. Evidence from the other sources mentioned proves adequate for profitable study and indicative of far-reaching conclusions.

The chief paleocartographical data used in these studies are: A Triassic continent, Gondwanaland plus South America (though the connection between Africa and South America may have been by way of Antarctica); a separation of Australasia from Malaysia, persisting since early Cretaceous times; Tertiary fluctuations in the Malaysian island connections, both with one another and with extra-Malaysian lands, which may have given brief connection between Papua and the southeastern Malaysian islands; a Cretaceous arm of the sea connecting the western Gulf of Mexico with the Arctic Ocean; a Cretaceous land strip from eastern Asia, running south of Alaska and down parallel to the western American coast and extending certainly to Central America and probably for a time to Ecuador or even to Chile (Tertiary connection of this strip was established with North America proper by the obliteration of the Gulf of Mexico-Arctic Ocean strait, and at the same time the southern portion of the land strip disintegrated); a Cretaceous or early connection of central or northern South America with Papuasia and Australia through the central Pacific islands; a bar to migration between southern Brazil and Patagonia, probably an arm of the sea or a strait, present in Cretaceous times or a little later and disappearing during the Tertiary, probably about the middle Miocene; an extended Antarctica, uniting Australia, New Zealand, Patagonia, sub-Antarctic islands in both Eastern and Western Hemispheres, and possibly Africa, the South American connection disappearing before the trans-Argentine sea was obliterated—Greater Antarctica was probably Cretaceous and persisted during the early Tertiary; an Indian Ocean continent, Lemuria, uniting Africa, Madagascar, Ceylon, southernmost India, the islands southwest of continental Malaysia and probably some of the southwestern Malay Archipelago islands (Lemuria existed in the Triassic, broke off from Africa during the Jurassic, and broke into separate islands before the end of the Tertiary). The data, discussed in the National Museum paper mentioned, agree with these paleocartographic conceptions, except for a single indication, namely, that there was a brief Tertiary connection between eastern Lemuria and continental Malaysia.

Let us note illustration of the handling of data in these studies. The Bell-toads evolved before the Cretaceous, since their spreading was not prevented by the Cretaceous separation of Australasia from Asia, they being present to-day in both Euro-Asia and New Zealand. They spread: North and west to Europe, where they are now represented; north and east to northern China (they are now present near the base of the Korean peninsula); south and east across Australia, where they left some of their characteristic