tory response to the subsequent administration of thyroid substance. The standard procedure described by Cutler, Power and Wilder[1] was used. The chloride concentration of a 4-hour urine specimen collected on the third day of chloride restriction in each of the 6 patients is shown in Table I. In each case, this value falls well within the range which has been found in control subjects. No significant change was observed in the serum concentration of chloride, the carbon dioxide combining power or the blood nonprotein nitrogen. None of the patients experienced any untoward symptoms during the period of sodium chloride restriction.

Conclusions. Six patients with primary hypothyroidism, in whom chloride excretion was studied during periods of sodium chloride restriction, showed no evidences of limitation of adrenocortical function.

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Dissociation of Pneumococcus by Radon Irradiation.

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Although extensive studies have been made on the bactericidal action of various rays emanated from radio-active substances[1] very little attention has been paid to the cultural and morphological changes that may follow such irradiation. Spencer[2] first called attention to the possibility of producing in a certain number of instances, distinct and stable morphological variants by exposing *Streptococcus hæmolyticus* and *E. typhi* to the *gamma* rays of radium. While some changes were noted in the cultural characteristics of these variants, few details were given. It has occurred to us that in addition to the morphological changes observed by Spencer, variations in cultural characteristics, serological specificity, and virulence might also result from irradiation. A comparison with another dissociant obtainable by the conventional method seems desirable. For these purposes, we have chosen the pneumococcus in our studies as the dissociants of pneumococcus are well known and readily ob-

1 Spencer, R. R., *Public Health Report* (Wash., D. C.), 1934, **49**, 183.
2 Spencer, R. R., *Ibid.*, 1935, **50**, 1642.

tainable. In the present communication, the effect of non-lethal *gamma* rays from radon tubes on Type I pneumococcus is described, and the dissociants thus obtained are compared with those produced by immune serum alone.

A virulent culture was plated and 10 colonies were picked in order to allow for the possible presence of some "radon-resistant" forms in the original culture. The organisms were grown in test tube 5.5 x 1.5 cm containing 2 cc liver-digest broth containing 1% glucose. After inoculation, radon tubes or bulbs of about 150 millicurie strength, encased in a brass capsule one mm in thickness, 6 mm in diameter and 30 mm in length, was placed in the same tube. In this way, *gamma* rays only were obtained. The tube was then incubated at 37°C. Every 48 hours, the culture was transferred into a new medium with the brass capsule containing radon tubes or bulbs. Every 24 hours the radon in tubes or bulbs was replenished to the initial strength of about 150 millicurie. Plates were made every day and colonies carefully studied. Occasionally, the culture was found to be contaminated, then the transfer was continued by picking some colonies from the plate made from the last irradiated culture. At the same time the following parallel experiments were run: (1) Culture with brass capsule without radon (control), (2) Culture grown in broth containing 5% homologous immune serum, and (3) Culture grown in broth containing 5% homologous serum and irradiated with radon at the same time.

Results. As the results with 2 series of experiments were essentially identical, only one of them is described in detail. After the 2nd transfer, colonies of the irradiated culture were found to be larger and more convex and mucoid than the controls, but returned to normal after another 3 transfers. From then until the 10th transfer, only smooth colonies were obtained. Five of such from the 10th transfer were inoculated into broth and the organisms were found to be fully virulent. Suddenly after one more transfer, striking changes were brought about: the colonies were all small, dry and extremely rough, but were completely soluble in bile and agglutinable by Types 2 and 3 sera. Twenty out of about 200 colonies obtained from the 11th irradiated culture were transferred to broth and tested for virulence. It was found that the sedimented organisms derived from as much as 20 cc of broth culture failed to kill white mice. The surviving animals were susceptible to the virulent organisms. These dissociants remained stable after weekly transfers for at least 6 months. At no time was a smooth colony observed in any of the subcultures. Attempts to revert 10 such colonies by the

mouse-method[3] were negative. It thus appeared that the dissociation was complete. Similar results were obtained with the second series, the only difference being that this sudden change in cultural characteristics and virulence took place after the 13th transfer.

The result from the control experiment was as follows: No change was observed with cultures containing brass capsule only, thus ruling out the possible dissociative effect of brass. The cultures grown in 5% immune serum broth also dissociated into rough forms. The following differences, however, were noted: (1) The pneumococcus grown in immune serum showed gradual changes from S to R while that seeded in the presence of radon irradiation exhibited sudden changes. (2) The loss of virulence of the serum-treated organisms appeared less complete. Even after the 20th transfer, 2-3 cc of broth culture could kill mice from which fully virulent pneumococcus could be recovered. In the presence of immune serum, a fewer number of transfers and a shorter period of irradiation were required to bring about dissociation by radiation. Thus in one experiment, only 5 daily transfers were necessary to produce 95% rough colonies from the radiated cultures. They possessed all the characteristics of those produced by radon alone.

From the above observations, it seems that irradiation with *gamma* rays obtained from radon can regularly induce a change of smooth to rough form of pneumococcus so complete that it was impossible to revert the dissociants to the primary state. Furthermore, by a combined action of immune serum and irradiation, it has been possible to achieve the same results but in a much shorter period of irradiation. This latter observation may offer a plausible explanation of the mode of therapeutic action of X-rays in cases of pneumococcal lobar pneumonia.[4]

Summary. Type I pneumococcus could be dissociated by *gamma* ray radiation. The rough forms appeared suddenly, becoming totally avirulent and irreversible. This dissociative change could be achieved in a shorter period of irradiation by the addition of immune serum.

[3] Griffith, F., *J. Hyg.*, 1928, **27**, 113.

[4] Scott, W. R., *Radiology*, 1939, **33**, 331.