worthy. In experiments directed toward the preparation of therapeutic anti-*H. influenzae* serum, Alexander and Heidelberger(17) found it necessary to extend the hyper-immunization over several months; they gave their rabbits multiple courses of injections each consisting of 4 daily doses of vaccine weekly for 4 weeks. A similar schedule was employed by Eaton *et al.*(18) for large-scale commercial production of antiserum. Both groups of workers noted(17,19) an appreciable mortality rate among the rabbits undergoing immunization. While some of the deaths were undoubtedly the result of the bleeding procedure and others were presumably due to intercurrent infections or other adverse conditions in the animal colonies, it is not unlikely that a portion of the deaths were due to the toxicity of the vaccines, since Dubos(20) has shown that these organisms may produce lethal soluble substance *in vitro*.

Our limited data on the preparation of anti-*K. pneumoniae* sera in chickens confirm those obtained for *N. meningitidis* and *H. influenzae*. It is highly likely that similar antisera can be readily prepared for other antigenic types of *Klebsiella pneumoniae* and *H. influenzae*, as well as various other encapsulated organisms. The successful results to date have been obtained with organisms the type-specific capsular antigens of which appear to be predominantly carbohydrate in nature. Whether Quellung would be demonstrable with non-carbohydrate capsular antigens is at the moment problematical.

*Summary and conclusions.* 1. Antisera satisfactory in the Quellung reaction were prepared for *K. pneumoniae* types A and B by injection of adult chickens with formalinized suspensions of the encapsulated bacilli. As few as 4 injections at weekly intervals were sufficient for this purpose.

2. Effective antiserum was similarly prepared for *H. influenzae* type B. Capsular swelling was readily demonstrable with sera collected from chickens after 3 or 4 weekly injections of living organisms from young cultures, while sera from rabbits undergoing the same immunization schedule were distinctly inferior at this stage.

3. Chickens are very useful for the production of Quellung antisera, with particular advantages where Gram-negative bacteria are concerned.

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## Effects of Phosphorus$^{32}$ on the Hamster. (18029)

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Phosphorus$^{32}$ was used in therapy as early as 1936. Later, Lawrence, Scott and Tuttle (1) employed it in a study of chronic leukemia and recommended its clinical use. Erf(2) attested its use in treatment of polycythemia. At the present time it is the treatment of choice for many cases of polycythemia. Dosages of phosphorus$^{32}$ sufficient to bring

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about complete symptomatic relief or remission of polycythemia may conceivably produce histological damage to sensitive tissue. The Histological Group of the Metallurgical Laboratory (Plutonium Project) at the University of Chicago reported considerable damage to the testes of mice treated intraperitoneally with 2.5$\mu$c of phosphorus$^{32}$ per g of body weight(3).

Results of a preliminary study of the effects of phosphorus$^{32}$ on the hamster are presented in this paper. The hamster was chosen as the test animal because of its possible use in future histological and radiogenetic research. Since there is little, if any, information available in the literature on the physiological and histological effect of irradiation on the hamster, an attempt was made to ascertain the toxicity of phosphorus$^{32}$ for the hamster and its effect on the histology of the testis.

*Experimental.* Ten hamsters from 3 to 5 months of age were used. They were descendants of a pair of the Bear Strain, obtained from the General Biological Supply House. They were fed Purina Hamster Chow supplemented with greens, carrots and potatoes. The animals were not crowded and were in a healthy condition at the beginning of the experiment. Two of the 10 animals were used as controls. The remaining animals, in pairs, were injected intraperitoneally respectively with 1, 6, 8 and 10 $\mu$c of phosphorus$^{32}$ (in the form of disodium phosphate) per gram of body weight. Animals treated with 1 and 6 $\mu$c/g were sacrificed after 24 hours and 30 days, those treated with 8 $\mu$c/g were sacrificed after 30 days, while those treated with 10 $\mu$c/g succumbed to the treatment in 11 days. The testes were taken intact from the animals with epididymis attached and fixed with Zenkers fluid overnight, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Tissue for autoradiographs was fixed overnight in 95% alcohol.

Damage to the testis from 1 $\mu$c of intraperitoneally injected phosphorus$^{32}$ per g of body weight was questionable at 24 hours. At 30 days there were occasional seminiferous tubules which consisted only of a basement membrane and Sertoli cells. Destroyed cells and apparently undamaged cells of all types were observed in almost all tubules. No alteration of the interstitial and Sertoli cells was noted. Autoradiographs showed a pale gray shadow throughout the entire organ 24 hours after treatment. Damage to the testis 24 hours after intraperitoneal injection of 6 $\mu$c of phosphorus$^{32}$ per g resembled that found 30 days after injection of 1 $\mu$c. However, a larger number of damaged tubules was observed. Thirty days after treatment with the larger dosage a marked decrease in the size of the testis was noted. Tubules were completely void of spermatogenic cells, but contained Sertoli cells and a loose protoplasmic network (Fig. 1, A). Roughly one per cent of the tubules contained sperm. Thickening of the basement membrane was prominent. Interstitial tissue appeared aggregated making it difficult to distinguish it from connective tissue. It is possible that some of the interstitial cells were affected. Damage to the testis 30 days after treatment with 8 $\mu$c of phosphorus per g was similar to that resulting from injection of 6 $\mu$c insofar as could be observed by histological examination, except that the protoplasmic network was lacking in the lumen of many of the tubules (Fig. 1, B). Damage to the testis 11 days after treatment with 10 $\mu$c/g of phosphorus$^{32}$ was questionable.

The general condition of the animals treated with 8 and 10 $\mu$c/g of body weight contributed information regarding the toxic effect of phosphorus$^{32}$ on the hamster. Both groups of animals showed radiotoxic symptoms indicated by loss of appetite, loss of weight and inflammation of eyes. Inflammation of eyes began 5 days after treatment and persisted through the eighth day. Recovery was evident in animals treated with 8 $\mu$c/g about the ninth day. By this time, eyes which had been closed since the sixth day were opening. Ten days after injection all symptomatic signs had disappeared. Animals treated with 10 $\mu$c/g showed no sign of recovery and both died on the eleventh day.

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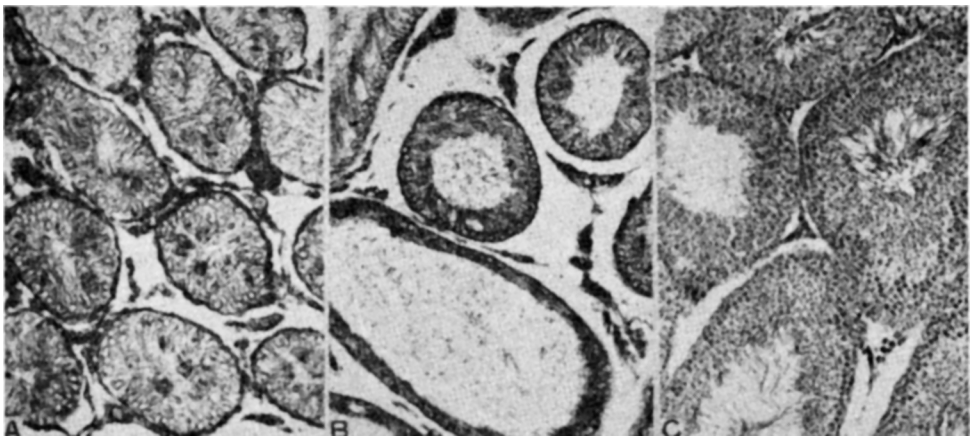

FIG. 1.
Sections through testes of 4-months-old hamsters. (A) 30 days after injection with 6 $\mu$c/g of phosphorus$^{32}$, (B) 30 days after injection with 8 $\mu$c/g of phosphorus$^{32}$, and (C) healthy controls. Note complete absence of spermatogenesis in both A and B.

The higher dosages of phosphorus$^{32}$ (6 and 8 $\mu$c/g) proved extremely destructive to the testis. Depopulation was complete in 30 days and there was no evidence of regeneration at this time. While the physiological and histological effects of irradiation are known to be dependent upon the time of exposure, it seems probable that a period of 30 days is sufficient for development of the maximum effects even with the lowest dosage of phosphorus$^{32}$ used. According to Schinz and Slotopolsky(4), regeneration of spermatogenic cells, if it takes place at all, begins before height of depopulation is reached. It is claimed by others(5) however, that restitution may begin 4 or more months after depopulation. Thus, although the results suggest that injection of 6 or more $\mu$c of phosphorus$^{32}$ per g is sufficient for permanent sterilization of the hamster, further work is needed to establish the exact dosage required.

*Summary.* Effects of intraperitoneal injection of the hamster with phosphorus$^{32}$ are described. Animals treated with 10 $\mu$c/g of body weight died in 11 days, there were no apparent changes in the testis at this time. Toxic effects were observed in animals injected with as little as 6 $\mu$c/g. Damage to the testes of animals treated with 6 and 8 $\mu$c respectively, was extensive, consisting of marked decrease in size, absence of spermatogenic cells in tubules, and actual damage to the tubules themselves, suggesting permanent sterility. No changes in the testes of animals treated with 1 $\mu$c/g were apparent in 24 hours. However, some damage was observed 30 days after treatment with 1 $\mu$c. Spermatogenic cells were more sensitive than Sertoli cells. Some interstitial cells appeared to be affected.

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