another portion, 0.1 ml. of propylene glycol only; and the final group, only the NaH_2PO_4. The eggs were sealed with sterile paraffin, marked, and placed in a modified Buckeye incubator with temperature and humidity control. Thirteen-day embryos proved convenient for analysis with a relatively high (40 per cent) survival rate.

The viable embryos were lifted with sterile forceps, cleaned of adhering membranes, and dropped immediately into liquid air. They were ground to a powder in a chilled mortar and the powder extracted several times with cold 10 per cent trichloroacetic acid followed by extracts with cold 5 per cent acid. The scheme of separation followed, with some modifications, that of Hevesy, *et al.* (*2*). The acid extract was filtered into cold, concentrated sodium hydroxide. The resulting solution was divided into three parts. From one aliquot we determined the average acid-soluble phosphorus compounds;

TABLE 1

Fractions	Activity: $\left(\frac{\text{Average counts/}\mu\text{g of phosphorus}}{\text{Counts from 0.1 ml. of original } NaH_2PO_4 \text{ solution}} \times 10^{+5}\right)$		
	Group receiving P* and vitamin D	Group receiving P* and propylene glycol	Group receiving P* only
Average acid soluble	0.530	0.522	0.633
Inorganic P	6.03	19.70	5.69
Adenosine P + inorganic P	8.12	4.00	2.18
Creatine P + inorganic P	4.15	3.64	3.32
Phosphatide P	2.33	1.65	0.825
Residual P (nucleoprotein)	3.02	2.10	0.285

P* = P^{32} in NaH_2PO_4.

the second aliquot was precipitated with 25 per cent barium acetate at pH 6.5, the precipitate was washed with dilute barium acetate, centrifuged, and a part dissolved in cold nitric acid. The solution was treated with ammonium molybdate reagent. This precipitate consisted of the inorganic phosphorus. The remainder of the barium acetate precipitate was ashed and the phosphorus determined. This fraction consisted of inorganic phosphorus plus adenosine phosphorus. The third aliquot was hydrolyzed with normal hydrochloric acid and 0.1 normal ammonium molybdate for 30 minutes at 40° C. The phosphorus released from the organic compounds precipitated. This fraction consisted of inorganic phosphorus plus phosphocreatine. The residue from the acid extractions was treated with an alcohol-ether mixture. The filtrate contained the phosphatide phosphorus, and the residue gave the so-called residual phosphorus containing mainly nucleoprotein phosphorus.

The phosphorus was determined in a Coleman junior spectrophotometer at 650 mμ, according to the procedure of Kitson and Mellon (*3*).

A summary of our results is given in Table 1. Detailed analyses for each embryo and for anatomical parts of 18-day embryos will be presented at a later date.

Our data reveal that the group of 6 embryos receiving the P* and vitamin D shows a higher specific activity for all fractions except inorganic P and the average acid-soluble P than the group of 5 embryos which received P* and propylene glycol and the 2 embryos which received only P*. The inorganic phosphorus value for the second group is inordinately high, but we can discover no contamination or error. These average values support the contention that vitamin D accelerates the over-all metabolism of inorganic phosphorus in the developing chick embryo. The effect is most marked in the adenosine, creatine, and phosphatide phosphorus fractions. Our data do not show any marked influence of vitamin D on the specific activity of the inorganic phosphorus fraction.

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Streptomycin Therapy in Experimental Tuberculosis of Guinea Pigs Infected Intracerebrally With Virulent Tubercle Bacilli[1]

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Up to the present time published data is not available concerning the effect of streptomycin in animals infected intracerebrally with virulent tubercle bacilli. The following is a preliminary report of such an experiment.

Twenty tuberculin-negative female guinea pigs weighing between 800 and 1,000 grams each were inoculated intracerebrally with 0.05 cc. of a culture containing 0.0001 mg. (dry weight) of a 7-day growth of H37Rv organisms in Tween-albumin liquid medium of Dubos (*1*). A small drill hole was made through the posterodorsal part of the skull, just to one side of the midline, and the organisms introduced by means of a tuberculin syringe and a ½″ No. 26 gauge hypodermic needle.

Ten animals were kept as untreated controls; the other 10 were started on streptomycin[2] treatment immediately after inoculation. They were given 4,000 μg at 7:30 A.M., 12:30 P.M., and 5:30 P.M., and 6,000 μg at 10:30 P.M., or a total of 18,000 μg/day. The drug was given either intramuscularly or subcutaneously.

During the third week after infection, both treated and control animals began to show signs of brain damage manifested by paralysis of the hindquarters, loss of equilibrium, and occasional convulsions upon stimulation. The control animals began to succumb on the 17th day, and all except one were dead by the 22nd day. The latter animal died on the 92nd day and developed increasing paralysis of the hindquarters for one week prior to death.

[1] This work was done under a grant-in-aid from the U. S. Public Health Service through the American Trudeau Society Streptomycin Program.

[2] Streptomycin was supplied through the courtesy of Dr. James Carlisle, of Merck and Company, Rahway, New Jersey.

On the 22nd day, at which time most of the treated animals were exhibiting the signs described above, the dose of streptomycin given to them was doubled, so that the animals received 36,000 μg/day. Within a few days the treated animals began to show improvement. The convulsive attacks and paralysis disappeared rapidly, the loss of equilibrium more slowly. Except for two animals which died of intercurrent disease on the 5th and 10th days after infection, the treated guinea pigs all remained well thereafter. After the 58th day, streptomycin treatment was stopped in one-half the group (4 animals), the remainder continuing on treatment. Those animals deprived of streptomycin showed a gradual return of paralysis of the hindquarters, which became progressively worse until time of death. All 4 animals died from the 98th to the 132nd day after infection (an average of 58 days after treatment was stopped).

TABLE 1

Guinea pig No.	Days of treatment with streptomycin	Days of life
1	None	21
2	"	17
3	"	92
4	"	19
5	"	19
6	"	19
7	"	18
8	"	19
9	"	22
10	"	21
1	5	5*
2	10	10*
3	58	132
4	58	127
5	58	109
6	58	98
7	173†	Living
8	173†	"
9	173†	"
10	173†	"

* Died of intercurrent disease.

† As of September 3, still living and being treated.

The 4 guinea pigs which were kept on treatment are still alive and well 173 days after infection.

All the treated animals responded to 5 per cent Old Tuberculin when tested intracutaneously 40 days after infection. The 4 remaining pigs are still skin positive at the time of writing this report, but the intensity of the reaction is declining. A summary of the time of death in relation to days of treatment is given in Table 1.

This experiment demonstrates that streptomycin administered intramuscularly or subcutaneously can effect inoculation tuberculosis of the brain in guinea pigs and cause improvement in the peripheral manifestations of such a lesion.

Previous experience in this laboratory has shown that an inoculum of attenuated or heat-killed tubercle bacilli equivalent to the infecting dose used in this experiment is not sufficient to produce skin hypersensitivity in guinea pigs. The results set forth in this paper, therefore, indicate that multiplication of the infecting organisms must have taken place in the guinea pigs despite the presence of streptomycin, and that it was only when the mechanisms of acquired immunity of the animal came into play that the disease was held under control by the drug. Further data on this subject will be reported from this laboratory (*2*).

The intracerebral method of infection offers a rapid means of testing the *in vivo* effect of antituberculosis drugs.

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"Acid Phosphatase" Reactions in Peripheral Nerves[1]

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The methods developed by Gomori (*4*) to reveal phosphatases in tissues have produced striking differentiations and are important additions to histologic technique. There is, for example, no simpler or more specific method for demonstrating the axis cylinders of nerve fibers than the acid phosphatase method following acetone fixation. The interpretation of the reaction, however, has presented difficulties so far as the nervous system is concerned. Heinzen (*5*) compared the acid phosphatase activity in the central and peripheral stumps of a series of transected nerves by standard biochemical methods and found increased activity in both stumps as compared with normal nerves. Histologic sections prepared by the Gomori method, on the other hand, showed a feeble reaction in the peripheral stump at a time when both stumps exhibited great activity *in vitro*. He suggested that the peripheral stump had suffered greater loss in staining capacity than the central between the time of removal of the tissue and the mounting of the sections.

Bodian (*3*) found the acid phosphatase activity in central stumps of transected nerves twice that of normal nerves and that of peripheral stumps 6 times as great. These biochemically determined differences could not be seen in sections prepared by the Gomori method. Bodian and Flexner then showed "that every step in the preparation of the histological sections for histochemical study sharply reduces the phosphatase activity as determined with the biochemical method."

We have studied the Gomori acid phosphatase reaction in peripheral nerves which had not been subjected to all the insults of histologic technique. Acetone-fixed nerves were teased in 80 per cent alcohol and transferred to the glycerophosphate-lead reagent buffered at pH 4.8 with molar acetate. After 2–18 hours at 38° C. they were washed thoroughly in distilled water and the lead visualized with ammonium sulphide.[2] Under these conditions the peripheral stump of a cat's sciatic nerve 16 days after transection appeared to the naked eye to be as intensely stained as the central stump. Under the microscope the normal fibers of the latter showed the usual precise staining of the axones; as in the normal controls, nuclei could rarely be demonstrated. Peripherally, where invasion of regenerating fibers had been prevented, all

[1] Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

[2] Rinsing with 1 per cent aqueous acetic acid before the sulphide did not materially alter the picture.