plague bacilli. As might be expected, 200 to 400 μg of streptomycin hydrochloride or sulfate, given every 6 hours, effectively cure 90 to 95% of the infections. Smaller doses or delayed treatment reduces the chance for cures (Fig. 2). Sulfadiazine in combination with antiplague serum is less effective than streptomycin (Fig. 3). The remarkable bactericidal action of streptomycin is fully documented by periodic bacteriological autopsies of treated and untreated mice (Fig. 4). Six hours after treatment with 200 μg had begun, the number of plague bacilli found in the lungs of the treated mice was reduced to approximately 60,000, while in untreated animals it had advanced to 10,000,000. By the 12th to 24th hours of treatment, the spleen became sterile and 5000 or less organisms were counted in the lungs. By the 96th hour after infection, when all untreated mice had died, the lungs and bronchial lymph nodes of treated mice were either sterile or contained only a few thousand plague bacilli in the abscess-like patches of pneumonia. No plague bacilli have been isolated from lungs or lymph nodes 100 hours after treatment with streptomycin. These results fully attest to the remarkable therapeutic efficacy of a total of 5 mg of streptomycin in experimental plague of mice. They justify an expectation that it will be equally effective in human pneumonic plague if administered early and in adequate dosage.

(5) *Suggested schedule of treatment in human plague.* Streptomycin is the most effective therapeutic agent thus far discovered for the treatment of bubonic, septicemic and pneumonic experimental plague infections in mice and guinea pigs. It is recommended that human plague be treated as soon as diagnosed with daily doses of 2 g of streptomycin in bubonic plague, and 4 to 6 g in the septicemic and pneumonic diseases; injections should be given at 4-6 hour intervals for the first 2 days. The dose may then be reduced, but in order to prevent clinical recurrences treatment should be continued for at least 8 days on a one g level or substituted with adequate sulfadiazine therapy. In profound toxemia, simultaneous administration of a potent antiplague serum, to assist the immunity mechanism, may prove beneficial.

Summary. Streptomycin in the amounts of 0.4 to 4.0 μg/cc is bactericidal for different strains of *P. pestis* in 5 days. Advanced experimental bubonic plague in mice is completely cured with 500 μg/3 hours for 3 days, or a total of 120 mg. Between 80 to 90% of the mice in a state of septicemic plague may be saved with a total of 1,200 to 1,600 μg. The remarkable bactericidal action of streptomycin is best demonstrated on experimental pneumonic plague; 5 mg of the antibiotic sterilize lungs and lymph nodes within 100 hours after treatment has been instituted. It is recommended that human plague be treated as soon as diagnosed with 2 to 4 g of streptomycin daily depending on the state of infection.

16147

Distribution of P^{32} in Incubated Egg.*

FRANK J. DIXON. (Introduced by Shields Warren.)
From the Laboratory of Pathology of the Harvard Cancer Commission, Harvard Medical School, Boston

As part of our observations of incubated eggs injected with P^{32} we traced the course of the isotope within the components of the egg throughout the incubation period. The phosphate content of a hen's egg of this size is approximately 220 mg[1] and the amount

* Work done under contract from Office of Naval Research.

of phosphate injected in this procedure was 3.5 mg.

Materials. Eggs—White Leghorns average weight 60 g incubated at 38°C with 70-80% relative humidity. P^{32} as KH_2PO_4—a 10% solution of the salt in H_2O was used for injection. The P^{32} was supplied as KH_2PO_4 by Clinton Laboratories, U. S. Government, Oak Ridge, Tenn.

Procedure. Approximately 0.05 cc of 10% KH_2PO_4 solution containing 20 μc per 0.05 cc was injected into the yolks of fertile eggs on the 5th day of incubation. One or 2 of these eggs were analysed daily during the remaining 15 days of incubation to determine the location and chemical combination of their P^{32}.

The analysis consisted of first separating the embryo, allantoic and amniotic fluid, yolk, albumen, and shell. Because of technical difficulties the allantoic and amniotic fluids were used together and not separated. The fluids were measured with a pipette or a tuberculin syringe and estimated to the nearest 0.5 cc. Greater accuracy in determination of these fluid volumes was not possible because of some mixing of the fluids during separation. The embryo and shell were weighed. Second, the radioactivity of these various components was measured with a Geiger counter. In order to avoid any errors due to selfabsorption, the embryos were completely ashed before measurement; the shells were dissolved in HCl and an aliquot of this solution was used for measurement. Third, in the eggs from the 6th to the 14th day of incubation each of the three fluid components was divided into ether extract, trichloracetic acid precipitate, and water soluble fraction after precipitation with trichloracetic acid. This was done by mixing 2 cc of the fluid sample with 8 cc of H_2O and extracting with ether for 15 minutes. To the H_2O soluble fraction was added 5 cc of 20% trichloracetic acid. This was filtered. After evaporation the activity of each of these fractions was measured.

Results. Complete results are listed on the accompanying table. Within 24 hours after injection into the yolk, $\frac{2}{3}$ of the P^{32} had left the yolk and was found principally in the allantois and amnion. After the first day, the balance of labeled phosphate in the yolk remained constant for 4 or 5 days and then gradually diminished in amount until at hatching time about 5% remained in the yolk. During this period the volume of yolk had diminished to about half of its original volume.

Within 24 hours after injection, more than $\frac{1}{2}$ of the P^{32} was in the allantoic and amniotic fluids. Subsequently, the amount of radioisotope in these fluids decreased in proportion to the uptake by the embryo, until at hatching time they contained only 3-4%. The volume of these fluids was constant until the last 4 days of incubation when it decreased rapidly.

The embryo's content of P^{32} increased nearly in proportion to its gain in weight. Shortly after injection its concentration was about 1.5 μc/g of embryo, and this ratio was maintained until the last 6 days when the concentration was closer to one μc/g of embryo. At hatching time the embryo contained about 90% of the P^{32} and made up a little more than $\frac{1}{2}$ the total weight of the egg.

The albumen never contained more than 3% of the injected radio-isotope. As incubation continued, the drop in P^{32} more or less paralleled the decrease in volume of albumen.

The shell and its membrane contained only 1% of the P^{32} throughout incubation. This small amount of activity lies within the experimental error because of the obvious difficulty of separating shell from all fluid contents.

Thus it is apparent that within 24 hours following injection, a shift of P^{32} from yolk to allantois and amnion takes place. As the embryo grows, its rate of uptake of the phosphate from allantois and amnion is much greater than from the yolk.

In addition to the above analysis we determined for each fluid constituent the amount of P^{32} contained in the H_2O soluble fraction, the ether soluble fraction and the protein precipitate. The distribution of P^{32} within

[1] Needham, J., *Chemical Embryology*, Vol. II, p. 1199, Cambridge University Press, London, 1931.

TABLE I.
Distribution of P^{32} in Incubated Eggs.
(Approximately 5 mg KH_2PO_4 containing 20μc P^{32} injected into yolk 5th day of incubation.)

Age of egg	Embryo		Allantois and amnion		Yolk		Albumen		Shell and membrane.
	Wt in g	% of total activity	Volume in cc	% of total activity	Volume in cc	% of total activity	Volume or wt	% of total activity	% of total activity
6	0.4	3	13.5	64	20	29	17 cc	2	2
7	0.8	6	14	52	20	37	14 "	2	1
8	1.4	10	13	53	22	36	12 "	1	1
9	2.2	18	15	50	21	29	11 "	3	1
10	3.1	28	11	29	26	39	10 "	3	1
11									
12	5.8	58	14	21	14	19	8 "	1	1
13	8.1	65	10.5	12	16	19	11.5 "	3	1
14	9.3	71	13	12	20	17	7 "	.7	1
15	13.3	73	13.5	13	14	12	5 "	.8	1
16	16.9	73	11	16	14	7	3 "	.6	1
17	18.1	76	6.5	17	14	7	1 g		.8
18									
19	25.5	93	3	3	14	4	1 "	.3	.3
20	27.4	90	7	4	9	5	.5 "	.4	.6

these various fractions was relatively constant throughout our period of observation from the 6th to the 14th day of incubation. Ninety per cent of the P^{32} in the allantoic and amniotic fluids was found in the H_2O soluble fraction, 9.4% in the trichloracetic acid precipitate and 0.6% in the ether soluble extract.

In the yolk nearly all of the activity was in the H_2O soluble fraction, only 1.1% was found in the ether extract.

In the albumen 80% of activity was found in H_2O soluble fraction and 20% in trichloracetic acid precipitate.

Summary. The radioactive isotope P^{32} was used as a tracer to follow the course of phosphorus combined as KH_2PO_4 and injected into the yolks of incubating eggs. The distribution of P^{32} within the various components of the eggs was determined daily throughout incubation. The distribution in ether soluble, water soluble, and trichloracetic acid precipitable fractions was determined for the fluid components of the eggs from the 6th to the 14th day of incubation. From the 6th day of incubation to the day of hatching, the P^{32} in the embryo increased from 3% to 90%.

16148

Changes in Plasma Inorganic Phosphorus of Dogs Under Postural Restraint.*

JOHN G. CONIGLIO.† (Introduced by C. S. Robinson.)

Form the Department of Biochemistry, Vanderbilt University, Schools of Medicine and Nursing, Nashville, Tenn.

During the course of some routine experiments the observation was made that, in unanesthetized dogs restrained in the supine position, the inorganic phosphorus concentration of whole blood and of plasma decreased to a low value of 30-60% of the original amount in 2 hours, after which it increased, in some instances attaining a level about

* Aided by a grant to Vanderbilt University, School of Medicine, from the Bristol-Myers Company.

† The author wishes to express his indebtedness to Dr. Gladys R. Bucher for much helpful advice and criticism.