calculation demonstrates that what is being measured includes incorporation of the P^{32} into organic constituents of the cell. In experiment 2*a*, for instance, the P^{32} concentration of the medium is represented by 7×10^3 counts/min/ml. Taking a value of 50×10^{-4} mm^3 as the volume of a whole ameba, the concentration of P^{32} in the cell is represented by about 2×10^5 counts/min/ml. There is, therefore, more P^{32} in the cell, by a factor of 50–100 for the several experiments, than could be accounted for by complete equilibration with inorganic PO_4. As has been pointed out by others, it is difficult even with data of this type to exclude the possibility that the organic P turnover is limited by the diffusion of inorganic PO_4. However, it has been shown in the case of other cells that the total P uptake is very closely dependent on metabolic variables (*7*), and it has been argued that the initial step in the uptake of PO_4 by the cell is not diffusion across the surface but incorporation into organic compounds (*6*). The conclusion that the present experiments demonstrate a nucleus-dependence of actual P turnover by organic constituents of the cytoplasm is considered probable. Current experiments in which the relative incorporation of P^{32} into various fractions is being determined should provide a more decisive test, as well as closer identification to those processes involving P turnover that are nucleus-dependent.

Although the experiments indicate that metabolic mechanisms in the cytoplasm which can be measured by P^{32} uptake are nucleus-dependent, and that the nucleus itself does not turn over a significant proportion of the P, the question of the nature of the nucleus-dependence remains unanswered. The total P^{32} uptake in 24 hr has been measured. Does the nucleus itself participate directly in the process in some way that does not involve its handling the P, or are we dealing with an indirect, long-term function of the nucleus (*8*)? Examples of the latter would be mechanisms whereby the nucleus was responsible for maintaining the submicroscopic structure of the cytoplasmic systems, or mechanisms whereby the nucleus was essential for the replacement of enzymes that "wore out" during the experimental period. The question may be answered by experiments in which the P^{32} uptake is measured during various intervals after enucleation. If the nucleus participates directly, the effect of enucleation should appear shortly after enucleation. If we are dealing with a replacement mechanism, the effect of enucleation should be more pronounced with time.

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The Use of a Radioactive Isotope in Determining the Retention and Initial Distribution of Airborne Bacteria in the Mouse[1]

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In studies of experimental airborne infections, it is important to know the retained dose and the initial distribution of the inhaled material in the host. The classical technique of grinding tissues to determine their bacterial content (*1*) may be an unreliable measure of the viable organisms present. More recently, bacteria that have been radioactively "tagged" have been used in distribution studies following injection (*2*), as have aerosols of inorganic radioactive material following inhalation (*7*). We have employed aerosols of radioactively "tagged" organisms in determining the retention and initial distribution of *Pasteurella pestis* in mice.

A 24-hr culture of an avirulent strain of *P. pestis* was grown in heart infusion broth (Difco) at room temperature on a shaker operating at a rate of 96 oscillations per minute with a stroke of 3 in. Five ml of this culture was transferred to a 100-ml flask of fresh medium containing approximately 1 millicurie of radioactive phosphorus (P^{32}) as soluble phosphate, and incubated as before. The viable count of such a culture was $1–5 \times 10^9$ cells per ml. The organisms were collected by centrifugation (2,000 rpm), washed twice in heart infusion broth, and resuspended in the same medium to yield a concentration of about 1×10^{10} viable cells per ml. In this suspension, one radioactive count per second represented approximately 1×10^5 viable organisms. Additional washings reduced the radioactivity of the cells by uniform amounts (approximately 10%), presumably in consequence of leaching. Two washings were considered sufficient for this investigation.

Mice of the Namru strain (*3*) were exposed to aerosols produced by atomizing suspensions of "tagged" bacteria in the apparatus described by Leif and Krueger (*6*). The average particle diameter in the aerosol was approximately 1 μ, as determined with the particle size analyzer described by Goldberg (*4*).

In the first series of experiments, groups of mice 6–8 weeks old, of both sexes, were exposed to the bacterial aerosol for 20 min. Samples of the aerosol were collected by capillary impingers during the exposure to determine the concentration of radioactive material. Exposed animals were sacrificed within 30 min, skinned, and bisected just below the diaphragm. The thoracic portions

[1] This investigation was supported in part by grants from the Office of Naval Research, U. S. Navy.

[2] The opinions and assertions contained in this report are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or of the naval service at large.

of the mice were heated individually in a muffle furnace at 300° F for 24 hr. The temperature was then raised to 1,000° F for 2 hr, and the radioactivity of the ashed remains determined. Another series of animals was similarly exposed but ashed *in toto* so that total retention in the animal could be ascertained.

Computation of the respiratory volume of the mice as a function of body weight was based on Guyton's (*5*) data. The theoretical dose received by each mouse was calculated from the computed respiratory volume and the concentration of the aerosol according to the following formula:

$$\text{Dose in cts/sec} = \frac{(\text{Wt of mouse, g})\ (1.25)\ (\text{Exposure time})\ (\text{Cloud concentration})}{1{,}000}$$

The results obtained in these experiments, presented in Table 1, indicate that the average respiratory retention

TABLE 1

PERCENTAGE RETENTION OF RADIOACTIVE MATERIAL IN MICE EXPOSED FOR 20 MIN TO AN AEROSOL OF *P. pestis* "TAGGED" WITH RADIOACTIVE PHOSPHORUS (P^{32})

No. mice	Calculated dose in counts/sec/mouse	Retention in respiratory tree		Retention in entire body	
		Counts/sec	% retention*	Counts/sec	% retention*
72	6.8	1.9	28	...	..
72	58.0	12.9	22	...	..
72	20.1	6.7	33	...	..
96	12.5	3.4	27	...	..
24	14.0	...	..	13.7	98
24	33.4	...	..	25.6	77
24	15.8	...	..	13.0	82

* % retention $= \frac{\text{cts/sec}}{\text{calculated dose in cts/sec}} \times 100.$

of organisms inhaled under the conditions described was approximately 30% of the calculated dose per mouse. This value may be compared with the total retention, which appears to be over 80% of the calculated dose.

In subsequent experiments, groups of mice were exposed, sacrificed, and dissected to determine the initial distribution of the inhaled radioactive material. In these animals, the activity was individually determined in the head, lungs, and trachea, stomach, intestines, and the remaining thoracic and pelvic portions of the body.

TABLE 2

RETENTION AND DISTRIBUTION OF RADIOACTIVE MATERIAL IN MICE EXPOSED FOR 20 MIN TO AN AEROSOL OF *P. pestis* "TAGGED" WITH RADIOACTIVE PHOSPHORUS (P^{32})

No. mice	Distribution of inhaled material (percentage of total retained)						
	Head	Lung and trachea	Remaining upper half	Stomach	Intestinal tract	Remaining lower half	Percentage total retention
16	8	21	5	38	..	28	106
14	10	16	8	24	..	44	124
30	..	13	12	22	39	14	...
30	..	14	9	38	27	12	...

From the results obtained (Table 2), it appears that approximately 30% of the retained material is to be found predominantly in the respiratory tree, as compared with about 70% predominantly in the gastrointestinal tract. The total body retention, determined on the first two groups of animals, was nominally equal to the theoretically inhaled dose.

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The Intracellular Distribution in Rabbit Liver of Injected Antigens Labeled with I^{131} [1]

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The gross distribution of labeled antigens following their injection into animals has been studied by numerous workers. Azoproteins containing arsenic (*4*), iodoproteins labeled with I^{127} (*5*) or I^{131} (*13*), and intensely colored antigens obtained by coupling proteins with dyes (*10, 11*) have been found to be deposited chiefly in the organs of the reticuloendothelial system, particularly in the liver and in the bone marrow; these organs, as well as the lymphatic tissues (*3, 14*), are regarded as the most probable sites of antibody formation. By means of the fixation reaction between fluorescent antibody and deposited antigen, it was shown that the antigen undergoes a granular deposition in the cytoplasm (*2*).

In this paper we wish to report on the intracellular distribution of radioiodinated antigens in the morphological fractions of rabbit liver prepared by differential centrifugation. Rabbits were injected intravenously with 80–100 mg of radioiodinated ovalbumin or beef serum globulin containing about 10% iodine. The iodoproteins were prepared from crystalline ovalbumin (*9*) or beef serum pseudoglobulin (*6*) according to the procedure of Wormall (*15*); the KI_3 solution used had been permitted to equilibrate with carrier-free radioactive iodide. At various intervals of time (from 75 min to 48 hr) follow-

[1] This investigation was supported by a grant from the Division of Research Grants, U. S. Public Health Service.

[2] Public Health Service Predoctorate Research Fellow, National Institutes of Health.

[3] The authors wish to express their gratitude to A. C. S. Mitchell, Department of Physics, Indiana University, for his helpful advice and his cooperation in obtaining the radioiodine used.