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-

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TABLE 1

ANALYSIS OF LIVER HOMOGENATE FRACTIONS OF RABBITS INJECTED WITH I^{131}-OVALBUMIN

Rabbit No.	30			31		
Injected antigen weight:	87 mg			80 mg		
activity (cpm):	1,374,000			819,000		
Time killed after injection:	9 hr			75 min		
Whole liver:	Wet weight (g)	Dry protein (g)	Activity of dry protein (cpm/g)	Wet weight (g)	Dry protein (g)	Activity of dry protein (cpm/g)
	60.0	13.5	1,230	64.5	13.0	8,800
Fractions:	Dry protein (g)	Activity of dry protein (cpm/g)	(%)*	Dry protein (g)	Activity of dry protein (cpm/g)	(%)*
Nuclear fraction	2.20	1,470	19.5	2.22	6,870	13.4
Mitochondrial fraction	2.00	3,430	41.5	1.89	31.400	52.2
Submicroscopic particulate fraction	2.30	625	8.7	1.96	8,980	15.4
Final supernatant	6.27	611	23.1	6.04	2,630	13.9
Total	12.77		92.8	12.11		94.9

* Percentage of the activity of the whole liver homogenate.

ing the injection, the animals (which had been fasted for 10–18 hr) were killed by exsanguination; their livers were removed, passed through a Latapie mill, and the pulp so obtained was homogenized to 10% in 0.88 M sucrose (*S*), using a glass homogenizer.

A portion of the homogenate (40 ml) was centrifuged three times at 600 *g* to obtain the *nuclear fraction* (*N*); centrifugation of the supernatant solution twice for 25 min at 20,000 *g* furnished the *mitochondrial fraction* (*M*). The *submicroscopic particulate fraction* (*P*) was obtained by diluting the supernatant solution to 0.25 M sucrose and centrifuging twice for 1 hr at 18,000 *g* (*12*). The first sediments of *M* and *P* were washed by resuspension and recentrifugation before being combined with the second sediments. The volumes of *N*, *M*, and *P* were brought to 25 ml, and the volume of the final supernatant (*S*) was between 200 and 215 ml. The entire fractionation procedure was performed at 0–5° C and required 6–7 hr.

In order to remove split products of the antigen, we added 4.25 ml of 40% trichloracetic acid to 20-ml aliquots of the original homogenate and of fractions *N*, *M*, and *P*, and 21.2 ml to a 100-ml aliquot of fraction *S*. Using 10-ml portions of the solvents, the protein precipitates were then washed by centrifugation and resuspension three times with 7% trichloracetic acid, twice with acetone, three times with ethanol, and again twice with acetone. The residues were dried at 55° C, weighed, pulverized, and spread evenly on aluminum planchets for counting by an end-mica-window Geiger tube. The sample thickness was consistent for the samples within each experiment and never exceeded 10 mg/cm^2, where only 5% of the activity was self-absorbed.

The activity of the iodoproteins injected into 7 rabbits varied from 700,000 to 3,000,000 cpm. The results of two typical experiments are presented in Table 1. The mitochondrial fraction had not only the highest activity per g of dry protein of any fraction, but it also accounted for a larger portion of the total activity than any of the other 3 fractions of the liver homogenate. In all our analyses, we found that 31.9%–69.8% of the total activity of the liver was located in the mitochondrial fraction. The activity of the nuclear fraction, although lower than that of the mitochondrial, was considerable. That this activity was not due to contamination with mitochondria was shown by the fact that the activity of the nuclear fraction of a rabbit injected with iodobeef serum globulin increased from 8,530 cpm/g to 9,280 cpm/g when the fraction was rehomogenized with 0.88 M sucrose solution and resedimented as described above.

In order to evaluate the extent of nonspecific adsorption of iodoprotein to the fractions, we added 0.04 ml of 3.46% iodobeef serum globulin to 100 ml of a normal rabbit liver homogenate and fractionated the mixture in the usual manner. We found 7% of the added activity in the nuclear, 3% in the mitochondrial, and 83% in the supernatant fraction containing the submicroscopic particles.

An analysis of the activity of various organs of rabbit No. 30 gave the following results: liver 1,860, spleen 1,250, bone marrow 2,800, lung 171 cpm/wet g, and urine 6,000 cpm/ml. No activity was precipitated from the urine by trichloracetic acid, showing that its high activity was due to split products of the antigen. When whole liver homogenates of this same animal were precipitated by trichloracetic acid 1, 3, and 7.5 hr after homogenization, the activities of the protein precipitates were 1,860, 1,890, and 1,810 cpm/wet g, indicating that no appreciable autolysis had taken place in the chilled homogenate.

The fact that the bulk of the deposited antigen is found in the mitochondria indicates that these particles

are involved in the formation of antibodies. As has been emphasized elsewhere (*7*), antibody formation can be interpreted as protein synthesis modified by the presence of antigen molecules. Actually, mitochondria have been considered to be endowed with the property of self-duplication (*1*), which implies protein synthesis.

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The Differential Induction of Lethal Mutations by Formalin in the Two Sexes of *Drosophila*[1]

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Several studies have been made recently in an attempt to administer chemical mutagens to eggs and sperms at various stages in ontogeny, followed by tests to detect variation in the mutation rate. It was shown that, whereas sex-linked recessive lethal mutations were induced in both eggs and mature sperms of adult *D. melanogaster* after vaginal douches with the N mustard methyl bis (ß-chloroethyl) amine hydrochloride (*6, 7*), there was no increase in the number of such mutations in either type of gamete when larvae were exposed to sublethal concentrations of this substance in the food (*8*). On the other hand, vaginal douches with formaldehyde failed to increase the mutation rate in eggs and mature sperms (*7*) but, although female larvae were not studied, large numbers of lethals occurred in the sperms of male larvae grown on food containing this chemical (*3, 9, 11*). Auerbach (*3*) has reported that formaldehyde fails to induce mutations in either type of gamete, mature or immature, when this substance is administered directly. She has presented evidence suggesting that an active mutagenic agent is produced only when formaldehyde is first mixed with the food. The ability of a chemical substance to induce mutations with some techniques and not with others may have several possible explanations (*1, 4, 7*). Such factors as the solvent for, the concentration of, and the duration of treatment with the chemical substance used may account for the results obtained with different techniques.

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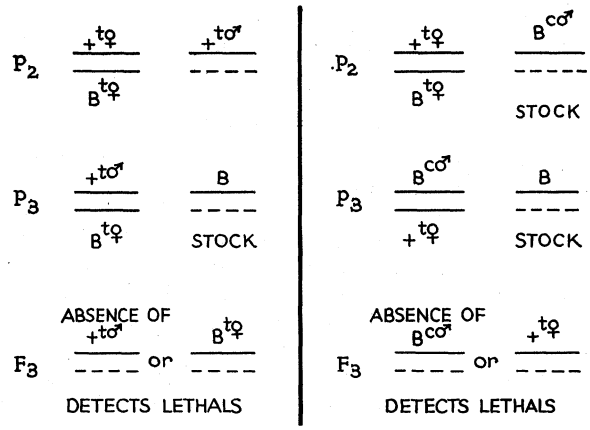

FIG. 1. Plan of matings to detect X-chromosome recessive lethals in progeny from P_1 crosses of Ore-R ♀♀ (+/+) by M-5 ♂♂ (B/Y). All ♀♀ virgin; all matings but P_1 in single pairs; solid line = X, broken line = Y chromosome; t = treated, c = untreated chromosome; ♀ or ♂ = P_2 parent from which chromosome came; up to 10 P_3 matings from each P_2 cross.

In the experiments reported here, *D. melanogaster* Oregon-R wild-type ♀♀ were crossed to Muller-5 ♂♂ and permitted to oviposit for 2 days in bottles containing 50 ml of a standard culture medium. After removal of the parents, 0.75 ml of a 8.9–17.8% solution of formaldehyde was added by pipette on top of the food, and the F_1 ♀♀ and ♂♂ were permitted to complete their development. For the P_2, half the F_1 ♀♀ were crossed to F_1 ♂♂, the other half to stock (untreated) Muller-5 ♂♂. The detailed plan of matings to detect sex-linked recessive lethal mutations arising in the gonads of P_2 parents is presented in Fig. 1. These lethals, detected in the F_3 generation, are of the following 4 types: + chromosome from treated F_1 ♀♀ ($+^t$♀); B chromosome from treated F_1 ♀♀ (B^t♀); + chromosome from treated F_1 ♂♂ ($+^t$♂); B chromosome from untreated P_2 Muller-5 ♂♂ (B^c♂). Lethals were retested for confirmation (*7*). The results are presented in Table 1.

A total of only 10 lethals occurred in 4,493 X chromosomes tested from control males (B^c♂) and treated

TABLE 1

TYPE OF LETHAL

	$+^t$♀	B^t♀	$+^t$♂	B^c♂
No. lethals	3	4	57	3
No. X chromosomes tested	1,546	1,401	1,401	1,546