In a third experiment, three 40 cm × 50 cm enamelled metal dishes were each given a deposit of 0.5 μg cm^{-2} of DDT. Two of the dishes were placed in subdued light at about 20° C and exposed to a continuous flow of air of about 10 m.p.h. from a fan for 14 days, after which the dishes were washed with 500 ml. of ethanol and, after concentration, aliquots were spotted on planchets for radioassay. Recoveries were 33% and 26% of that obtained from the third control dish, which was washed as soon as the applied solution had dried. The mean rate of loss from the dishes was 1×10^{-3} μg cm^{-2} h^{-1}.

A further experiment was designed to recover and identify the volatilized radioactivity. Eight planchets were given an initial deposit of 0.5 μg cm^{-2} of DDT and covered by clean inverted planchets, which were kept apart by cardboard washers. The inverted untreated planchets acquired radioactivity with a mean count of 20 c.p.s. after 14 days at about 20° C. The radioactive contamination was removed by Soxhlet extraction with ethanol, and after concentration by rotary evaporation aliquots were spotted for thin-layer chromatography on silica-gel covered plates and run in cyclohexane: chloroform (80 : 20). When scanned, one radioactive peak was obtained with an R_F corresponding to that of the original DDT-C14. A similar result was obtained when the plates were run in hexane.

The experimentally determined evaporation rates were in good agreement with the values predicted by the two methods of calculation which, converted to a field scale, suggest losses at the rate of about 2 pounds acre^{-1} yr^{-1} in summer and about 0.3 pounds acre^{-1} yr^{-1} in winter. The interesting implication is that about half the DDT applied to field crops may enter the atmosphere.

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Of Mice and Means

The use of control and experimental groups of animals for the study of *in vivo* effects of drugs and foreign chemicals is a standard practice. Considerable care is given to several accepted precautions such as the establishment of identical conditions for both groups and the choice of litter mates of the same sex. If a drug is to be given parenterally to the experimental group each corresponding control animal must receive an injection of equal volume and ionic composition given by the same route. Less attention is usually paid to the segregation of the animals during the experiment. A common method is for several animals to be kept in a number of separate cages. The first animal taken from each cage may be used for control purposes and the remainder given different doses of the drug. Alternatively either one cage of animals may be designated the control group and other cages used for a particular drug dose or the control and experimental animals may be distributed randomly throughout the cages. In all these procedures it is implicit that the order in which the animals are used should not affect the biological system being measured. We have obtained some results which suggest that this assumption is not justified.

It was noticed in the course of some work concerned with the incorporation of radioactivity from labelled amino-acids into the protein of mouse liver that there was a trend in the results obtained with the control animals. This trend seemed to depend not only on the order in which individual animals were taken from a particular cage but also on the order in which the cages were used. Further experiments were therefore made, in each of which three cages, each containing four mice, were used. The animals were given an intraperitoneal injection of 0.5 ml. of 1.3% NaCl solution at 4 min intervals and immediately transferred to individual containers. The order in which the animals were taken out from each cage and the order in which the cages were used were carefully noted. After exactly 1 h each animal was killed by cervical fracture, the liver was removed and the incorporation of ^{14}C-histidine into the proteins of the post mitochondrial fraction determined by standard methods[1].

The results (Table 1) show that the incorporation of radiocarbon into the isolated proteins decreased both with the order in which the animals were taken out of the cages and with the order in which the cages were used. There are statistically significant differences between the results obtained with the first and last animal removed from each cage and between those measured in the animals originally housed in the first and third cages. Similar effects were also observed in experiments in which labelled amino-acids were injected into intact mice.

Table 1 Incorporation of ^{14}C-Histidine into the Proteins of the Post Mitochondrial Fraction of Mouse Liver

Order of removal of animal from cage	^{14}C incorporated (c.p.m./mg protein)	Order in which cages were used	^{14}C incorporated (c.p.m./mg protein)
1	344 ± 105 (10)	1	352 ± 72 (14)
2	284 ± 69 (9)	2	277 ± 76 (13)
3	271 ± 71 (10)	3	232 ± 45 (12)
4	261 ± 61 (10)		

The results are given as means ± s.ds., the number of animals used being shown in parentheses. They have been analysed by the *t*-test and a statistically significant difference ($P < 0.05$) was found between the first and last animals taken from each cage and between the animals in the first and third cages.

One implication of the present work concerns the interpretation of the results of experiments designed to reveal the possible effects of an agent on protein biosynthesis in the mouse. If either the first animal from each cage or the first cage were chosen as controls, then an inactive substance may have yielded spuriously positive results. This error would have been compounded if the first animals were control and the subsequent mice received increasing doses of the test material. The opposite could also occur in that the effect of an inhibitor could be masked if the last animal from each cage or the last cage were the controls. Although our experiments have been restricted to the determination of one aspect of protein synthesis in mice the possibility that similar effects occur with other biological parameters in laboratory animals normally maintained in small groups in cages must be considered. Any experimental design should take into account the possibility that the order in which the animals are separated into control and test groups may influence the results independently of the agent or procedure being investigated.

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