by the method outlined by Gray(6).

Seven of the 30 implanted mice treated with cortisone died before the tumors reached appreciable size; 4 died on the eighth, 2 on the ninth, and 1 on the eleventh day following the beginning of treatment. It is presumed that these mice died as a result of the large repeated doses of cortisone since 4 out of 18 normal unimplanted male CF_1 mice, similarly treated in experiments made to ascertain lymphoid tissue reducing effects, died between the seventh and tenth day of treatment. Normal mice receiving 40 mg/kg of cortisone acetate subcutaneously for 10 consecutive days show at autopsy profound atrophy of the thymus, reduction in the size of the spleen and considerable loss of weight.

Discussion and summary. The data show that the resistance of alien strain (CF_1) mice to the progressive growth of lymphosarcoma 6C3H-ED (originating in C3H mice) is greatly impaired by intensive treatment with cortisone acetate. Seventeen of the 23 CF_1 mice which survived treatment with cortisone died of progressive growth of lymphosarcoma implanted from C3H mice, whereas only 2 of 37 untreated mice developed progressive tumors.

It should be pointed out that the course of cortisone dosage used to bring about the effects described were excessive, actually constituting a lethal concentration for some of the CF_1 mice. The nature of the change in resistance shown by these experiments has not been established, but it might be inferred that it is associated with the well known depression of normal lymphoid tissue and immune responses which large doses of cortisone induce.

Addendum. After the above manuscript was submitted for publication, a paper by E. L. Howes (*Yale Jour. Biol. and Med.*, 1951, v23, 454) came to our attention. This paper described the progressive growth of adenocarcinoma EO771 or 775 when transplanted in Rockland Swiss mice treated with cortisone.

6. Gray, P., *Arch. Biochem.*, 1947, v13, 461.

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Effect of Roentgen Irradiation upon Protein Absorption in the Mouse.* (18903)

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In a study of the effect of X-irradiation upon fat absorption in the mouse Mead, Decker and Bennett(1) showed that contrary to most earlier reports, no impairment of absorption could be demonstrated and that the results of previous workers could often be explained by radiation-induced changes in motility of the gastrointestinal tract.

In a continuation of these experiments we have now investigated the effect of X-irradiation upon protein absorption. The purpose of this study was to determine first, whether the previous findings represented an isolated case or could be generalized to cover other types of nutritive elements; and second, whether the damaged small intestine might permit the passage of appreciable quantities of material with molecular sizes larger than those of the amino acids. Under ordinary circumstances in normal animals, proteins are completely broken down before absorption. However, evidence has accumulated which indicates that in the irradiated animal intestinal permeability may be altered so as to allow the passage of bacteria(2). It was thought that the possibility of the absorption of intact protein could readily be demon-

* This paper is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

1. Mead, J. F., Decker, A. B., and Bennett, L. R., *J. Nutrition*, 1951, v42, 485.

2. Hammond, C. W., and Miller, C. P., *Ann. New York Acad. Science*, 1950, v53, 303.

strated by the use of iodinated proteins, since the iodinated amino acids released by hydrolysis are not rapidly reincorporated into serum proteins(3).

Materials and Methods. Human serum albumin iodinated with I^{131}† was used as the test protein in this study. To a solution of this protein was added an appropriate amount of bovine serum albumin (Cohn's fraction V)‡ to make a solution containing 35-37% total protein. The animals used for the study were female mice of the C.F. 1 strain, which had a mean weight of 27.5 g with extremes of 22.0 and 34.3 g.

During the first of the experiment, it was thought necessary to keep the mice on a protein-free diet§ for 12 hours before use. However, some of the animals lost weight during this time; and when it was found that absorption was in the same range for animals on a commercial stock diet, this initial procedure was abandoned and the animals used subsequently were given no preliminary change in diet.

For the determination of the rate of protein absorption, mice were fed a weighed amount (about 0.2 g containing 55-80 mg of protein) of the iodinated albumin solution measuring about 10,000 c/sec. on the counter in use. This material was delivered well into the esophagus using a 0.25-ml syringe with a bent No. 19 hypodermic needle ending in a blunt silver-solder tip. After the appropriate time had elapsed, the mice were sacrificed with chloroform. The gastrointestinal tract was dissected out, divided into 3 segments, and flushed with saline, as described previously(1), into 100 ml volumetric flasks. The suspensions were shaken thoroughly and allowed to stand for at least 15 minutes, after which time they were diluted to the mark with saline, shaken again, and allowed to settle for 5 minutes.

The activity in each segment was determined in triplicate by counts of basified (with NaOH) dried 0.5-ml aliquots of the washings. The amounts of protein recovered were then ascertained by a comparison of the activities in these samples with those in similarly treated samples of a standard prepared by diluting a weighed amount (0.05-0.1 g) of the test solution to 50 ml. The amount of protein absorbed could then be calculated from the difference between the dose given and the total amount recovered. Completeness of recovery was substantiated in 2 mice sacrificed 5 seconds after administration of the dose, the recovery values being 104% and 97%.

Serum protein-bound iodine was determined by comparing the activity of 0.1-ml aliquots of serum which were diluted with 0.5 ml saline, made alkaline with NaOH and dried, with that of precipitates obtained by the action of 5% trichloracetic acid on 0.1 ml aliquots of serum from the same animal. The

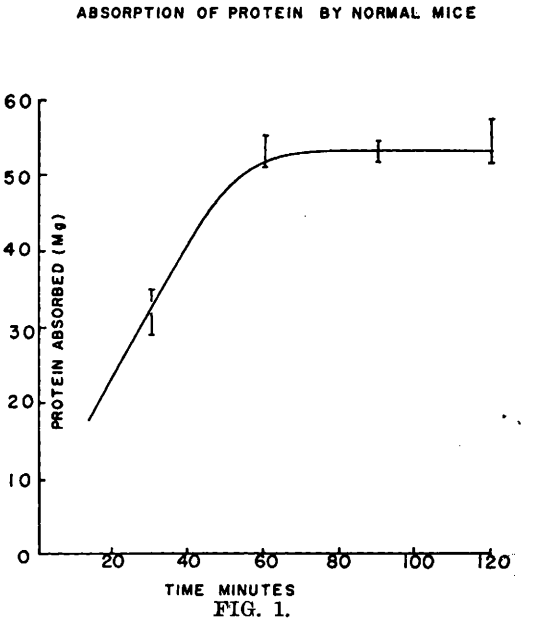

FIG. 1.

3. Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *Science*, 1951, v113, 274.

† Obtained as a solution from Abbott Laboratories. A micro-Kjeldahl determination made on one lot showed it to contain approximately 2% protein.

‡ Obtained from Armour Laboratories.

§ The diet consisted of 41% starch, 41% sucrose, 8% fish liver oil-corn oil mixture, 4% salt mixture (obtained from Nutritional Biochemicals Co.) and 6% celluflour (obtained from Chicago Dietetic Supply Co.). To condition the animals to the powdered diet, they were given the above diet with 20 g casein added per 100 g for two days previous to the protein-free diet.

TABLE I. Protein Absorption in Mice.

	No. of mice	mg protein in dose	% in stomach	Total protein absorbed in mg	% absorption of protein reaching small intestine
30-min test period					
Normal mice	7	55.8 ± 1.9	30.3 ± 5	31.9 ± 3.1	81.4 ± 2.2
2 hr post-irrad.	7	77.7 ± 2.4	53.5 ± 4.1	30 ± 2.6	84.1 ± 3.5
24 " "	8	79.3 ± 2.6	51.6 ± 5.8	32.4 ± 3.8	85.7 ± 3.3
96 " "	7	81.3 ± 1.6	41.8 ± 3.8	37 ± 3.6	77.6 ± 4.1
90-min test period					
Normal mice	10	79.1 ± 3.2	25.2 ± .9	53 ± 1.5	90.5 ± 1
2 hr post-irrad.	7	81.1 ± 1.4	39.8 ± 6.3	44.9 ± 5.1	91.4 ± 1.3
24 " "	8	78.4 ± 2.4	38.3 ± 5.2	44.7 ± 3.4	93 ± 1.5
96 " "	7	83.6 ± 2.9	28.3 ± 4.6	55.3 ± 4.6	92.2 ± .5

precipitates were centrifuged and washed 3 times with 5-10 ml portions of 5% TCA and were transferred to the planchets with 1.0 to 1.5 ml of saline and dried.

By means of the same technique, the standard was shown to contain about 98% protein-bound iodine. As a check on the adequacy of the precipitation and washing procedures, precipitations were carried out using normal mouse serum precipitated with the supernates remaining after the precipitation of sera from test animals which had received the iodinated protein. In each case, calculations were made of the percent of activity of the precipitate as compared with that of the whole serum.

Since a survey of the literature failed to reveal data on the rate of absorption of protein in the normal mouse, this investigation was undertaken as preliminary to that of the effects of X-irradiation.

Absorption was studied in normal mice at 30-minute intervals from zero to 120 minutes with a few points taken at 5 to 15 minutes. For the irradiated animals only the 30- and 90-minute periods were studied.

Irradiation of all mice was carried out at a distance of 50 cm and a dose rate of 41 r/min. for 600 r.‖ This dose is somewhat greater than the mid-lethal dose in these laboratories.

‖ The radiation factors were: 250KV machine, operated at 15 Ma., with 0.21 mm CU inherent, 0.5 mm Cu parabolic, and 1.0 mm Al filters; HVL in the center of the field, 2.0 mm CU; 50 cm focal object distance.

Results. The absorption of protein by normal mice is shown graphically in Fig. 1, absorption of the test meal has essentially ceased after 60 minutes, and no further absorption could be observed to occur even up to 120 minutes, the unabsorbed portion of the dose being recovered from the stomach.

In all the irradiated animals for both test periods there was a marked retention of the dose in the stomach at 2 and 24 hours post irradiation (Table I, column 4). By 96 hours post irradiation, the emptying time of the stomach was approaching normality.

Despite this effect on the stomach emptying time, total protein absorption was only slightly reduced (Table I, column 5), and if absorption is calculated on the basis of the protein actually reaching the small intestine, no significant difference can be seen between the irradiated and control animals (Table I, column 6).

In Table II are recorded the results of the determination of protein-bound iodine in the sera of normal and irradiated mice and that found by precipitating normal serum with the supernates from precipitation of radioactive serum. It can readily be seen that there is no evidence for large-particle absorption either in the normal or in the irradiated animals since the activity of the precipitates in the 3 cases is of the same order of magnitude and apparently represents a constant error due to adsorption of small active molecules by the precipitate.

Discussion. The adequacy of I^{131} as a tracer for protein has recently been attested by Dixon and co-workers(3). However, it

TABLE II. Distribution of I^{131} in Blood Serum.

	No. of mice	Mean % of counts in TCA ppt	Range
Non-irradiated mice	28	1	.5–2.3
Irradiated mice	24	1	.2–3.8
Normal serum ppt with TCA supernatant from a radioactive mouse	12	1	.2–2.8

could be argued with some truth that in this case, since no whole protein was absorbed, the only rates measured were those of hydrolysis of protein and absorption of iodotyrosines. It is possible that the different amino acids are absorbed from the mixtures at different rates(4). Nevertheless it was thought that in this case, the iodotyrosines could be taken as typical amino acids in their absorption behavior and that the appearance of radio-activity in the blood would be a good measure of amino acid absorption in general. If significant differences in the absorption rates of normal and irradiated rats were found, they could be tken as symptomatic of the general permeability of the small intestine. Since no such differences have been found, it can be concluded that as seen in the case of fat absorption(1) no permeability differences are produced in this animal by this dosage of radiation and that in this case as well, motility and tonicity are the factors most affected. These factors have recently been investigated by Conard(5), who, using direct observation of the externalized intestine, found marked changes in motility following irradiation.

The study has also shown that no large quantities of whole protein are absorbed in either the normal or the irradiated animals, and that the ability of the irradiated animal to absorb protein in the normal fashion is essentially unimpaired for the periods and dosages studied.

Summary. The rate of protein absorption by normal mice has been measured using radioiodinated human serum albumin as a trace protein. No change in this rate could be shown after whole body X-irradiation of the mice although a change in tone and motility of the gastrointestinal tract was noted. No absorption of unhydrolyzed protein could be demonstrated.

4. Orten, A. U., Koizumi, K., France, C. J., and Johnston, C. B., *Fed. Proc.*, 1951, v10, 390.

5. Conard, R. A., *Am. J. Physiol.*, 1951, v165, 375.

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Effects of N-allylnormorphine on Cholinesterases. (18904)

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Bernheim and Bernheim(1) found that morphine and apomorphine inhibit the cholinesterase of brain in sufficiently low molar concentration to enable them to speculate that this action of these drugs might be responsible for the cardiac and respiratory irregularities sometimes associated with their use. Wright and Sabine(2) confirmed the inhibitory effect of morphine on cholinesterase, and showed that Dilaudid and dihydrodesoxymorphine-D also inhibit the enzyme. They also reported that the effect of codeine varies considerably with the source of the enzyme (brain or serum). Eadie(3) reported that the inhibition of the cholinesterase of dog serum by morphine is a competitive one.

N-allylnormorphine (NAM) is a substance

1. Bernheim, F. and Bernheim, M. L. C., *J. Pharmacol.*, 1936, v57, 427.

2. Wright, C. I. and Sabine, J. C., *J. Pharmacol.*, 1941, v72, 45.

3. Eadie, G. S., *J. Biol. Chem.*, 1941, v138, 597.