related systems and to additional mutants of different types.

Summary. The ability of amino acid and of purine and pyrimidine auxotrophs of *Aerobacter aerogenes* to form adaptive enzymes for the degradation of *myo*-inositol has been investigated. It was found that adaptive enzyme synthesis by the amino acid auxotrophs could be stimulated in the absence of growth by addition of the required nutrilite, but not by addition of $(NH_4)_2SO_4$. In contrast, adaptation in the guanine and pyramidine auxotrophs was enhanced by addition of $(NH_4)_2SO_4$ alone. In all cases, dividing cells attained higher levels of adaptive enzyme than resting cells. The bearing of these results on the mechanism of adaptation is discussed. The poor utilization of histidine by strain 50 for growth on inositol does not seem to be due to the effect of histidine on adaptation, but rather seems to suggest a metabolic relationship between inositol and histidine.

1. Spiegelman, S., and Dunn, R., *J. Gen. Physiol.*, 1947, v31, 153.
2. Halvorson, H. O., and Spiegelman, S., *Bact. Proc.*, 1952, 151.
3. Magasanik, B., *Am. Chem. Soc. Abst. of Papers*, 119th Meeting, 20C, 1951.
4. ———, *J. Am. Chem. Soc.*, 1951, v73, 5919.
5. Magasanik, B., Brooke, M. S., and Karibian, D, *Bact. Proc.*, 1952, 164.
6. Davis, B. D., *Proc. Nat. Acad. Sc.*, 1949, v35, 1.
7. Clifton, C. E., *Enzymologia*, 1937, v4, 246.

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Response of X-irradiated Mice to Intravenous Inoculation of Intestinal Bacteria.* (19714)

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The possibility that infection by bacteria of intestinal origin plays a significant role in death following exposure to ionizing radiation has been suggested by a number of observations. Bacteria common to the lower intestinal tract have been found in blood and organs of animals after total body X-irradiation(1-4). Miller *et al.*(5,6) showed a high incidence of bacteremia identified with normal intestinal inhabitants in mice during the second week after exposure to 450 or 600 r total body X-irradiation. This was the period of greatest mortality. Furthermore, it has been possible to reduce mortality among irradiated animals by the administration of various antibiotics(7,8). The protective effect was considerably reduced in mice if there was a prevalence of invading intestinal bacteria which were relatively insensitive to the antibiotics(7). Increased susceptibility of mice to inoculation with *Escherichia coli* following sublethal total body X-irradiation was shown by Shechmeister *et al.*(9,10). Subcutaneous or intraperitoneal injection of *E. coli* caused much higher mortality among irradiated mice than among normal animals receiving the same inoculum.

The present experiments were undertaken to study the effect in mice of intravenous inoculation of another normal intestinal organism, a member of the genus *Proteus*, after moderate total body X-irradiation.

Experimental procedure. C_3H mice, 60-90 days of age, were subjected to total body X-irradiation in groups of 3 in plastic (Styron) containers. The radiation was delivered from a 200 kv Picker-Waite X-ray therapy machine operating at 20 ma and a distance of 20 cm. The filters consisted of 0.5 mm Cu and 1.0 mm Al. Doses ranged from 400 to 550 r, given at a rate of 230 r per minute. Non-irradiated controls were kept in the plastic containers for the same length of time as the irradiated animals. The mice were housed in plastic cages in groups of 6 in an air-condi-

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tioned room at 72°C and were maintained on a commercial stock ration.

Three days after irradiation the mice were inoculated in the tail vein with 2-5 million *Proteus* contained in 0.2 ml saline. Groups of animals given the same X-ray dose followed in 3 days by inoculation with 0.2 ml sterile saline and groups not irradiated but injected with 2-5 million *Proteus* were included as controls. All mice were observed for 30 days after irradiation to obtain mortality data. The bacterial inoculum was prepared by suspending the growth from several 24-hour agar slants in sterile saline, estimating the number of organisms from turbidity-plate count data, and diluting with sterile saline as required. A plate count was made on this suspension to check the number of organisms injected. The culture of *Proteus* used was obtained from heart blood of a mouse given X-irradiation only. When originally isolated, the organism had the biochemical characteristics of *Proteus vulgaris*. After having been maintained as a stock culture for over a year, its properties more closely resemble those of *Proteus morganii*. Sucrose and maltose are usually not fermented, although slow acid production is occasionally observed in these sugars. The organism no longer produces H_2S in lead acetate agar and attacks urea more slowly than when first isolated. Similar changes in apparent *P. vulgaris* cultures have previously been observed after maintenance in stock culture(11). Both *P. vulgaris* and *P. morganii* have been found in cecal content and feces of our mice.

In experiments at 500 and 550 r, tail blood samples were taken from mice in each of the 3 groups to determine the number and kind of bacteria present. Blood was taken within one minute after inoculation and daily thereafter for 15 days. The tail was dipped in tincture of iodine, allowed to dry, snipped with sterile scissors, and a measured amount of blood taken in a sterile red blood cell diluting pipette. Serial dilutions were made in 0.85% sodium chloride solution containing 0.5% sodium citrate. One-half ml amounts of various dilutions were spread over the surface of Levine's eosin-methylene blue agar (Difco) and Bacto heart infusion agar (Difco) plates. A small sample of whole blood taken in a capillary pipette was plated directly on these media. The tail was cauterized after taking the samples. Plate counts were made after 48 hours incubation at 37°C. Cultures for identification procedures were isolated at this time. Mice which were bled were not tabulated in survival data.

TABLE I. Effect of Intravenous Inoculation of *Proteus* on Mortality of X-Irradiated Mice.

X-ray dose	Inoculum	Mortality* Dead/ Total	Mortality* % dead	Median time of death (days post-x-ray)
400r	*Proteus*†	6/14	43	9.5
400r	Saline	0/12	0	—
500r	*Proteus*	20/20	100	6
500r	Saline	3/18	17	12
550r	*Proteus*	12/12	100	7
550r	Saline	5/12	42	16
None	*Proteus*	0/34	0	—

* Mortality data based on observation of mice for 30 days after irradiation.

† No. of *Proteus* inoculated ranged from 2–5 × 10^6.

Results and discussion. More deaths occurred among irradiated mice inoculated with *Proteus* than in control mice given the same irradiation followed by sterile saline injection. At 500 and 550 r the death rate was increased to 100% by *Proteus* inoculation. There were no deaths among non-irradiated animals given the same number of *Proteus*. At all 3 X-ray doses, the median time of death in days after irradiation was shorter for mice inoculated with *Proteus* than for those receiving sterile saline. The results are summarized in Table I. These data show an increased susceptibility to infection with a normal intestinal organism in mice subjected to moderate total body X-irradiation. Such heightened susceptibility to infection among irradiated animals has previously been shown with various pathogenic organisms(12,13) and *E. coli*(9,10). The increased number and earlier occurrence of deaths caused by injection of *Proteus* after moderate doses of X-ray support the concept that infection with intestinal bacteria is important in radiation death. These observations are evidence against the idea that infection is merely a terminal event in death following irradiation.

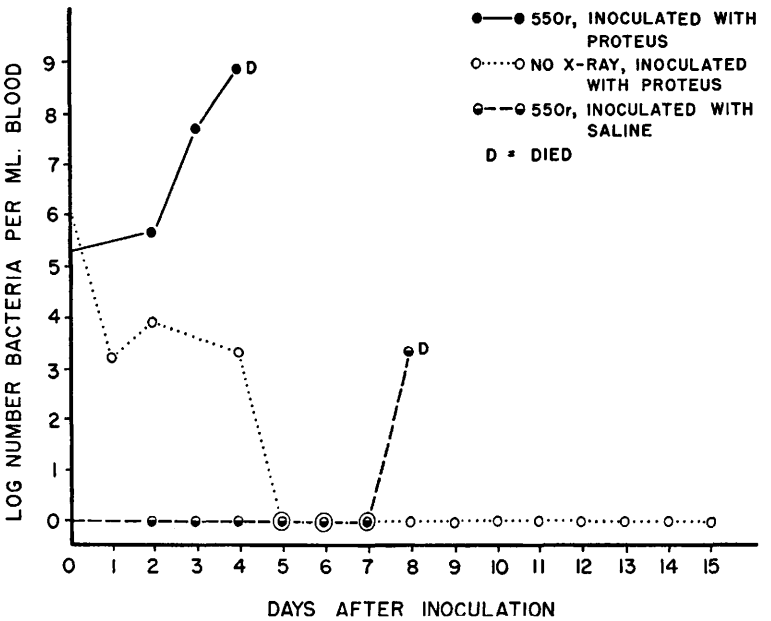

FIG. 1. Bacterial counts of blood of representative mice from an irradiation-inoculation experiment at 550 r.

The plate counts on daily blood samples indicate that the animals subjected to 500 or 550 r had little ability to restrict multiplication of the inoculated bacteria. The organisms increased in number until death occurred, generally rising to 10-100 million per ml blood. Apparently the defense mechanisms related to blood clearing were greatly impaired by irradiation. Chrom(2) observed reduced blood clearing capacity in irradiated mice injected with the Breslau bacillus (probably *Salmonella typhimurium*. The non-irradiated mice were able to remove the inoculated *Proteus* from the blood comparatively well. The number was reduced markedly during the first day after inoculation, but some were found in the blood for several days, usually only a few thousand or less per ml. By the 11th or 12th day after inoculation the blood was free of bacteria. The irradiated, saline-injected mice rarely showed any bacteremia until 7 or more days after inoculation (10 or more days after irradiation). Bacteria found in the blood subsequent to this time were *Proteus* species, unidentified streptococci, and, on one occasion, a *Pseudomonas*. These organisms were presumably invading from the intestine. Both the types of bacteria found and the time of occurrence of bacteremia were similar to the findings of Miller *et al.*(5). At 550 r all mice which died in the group injected with saline and repeatedly bled showed bacteremia before death. However, at 500 r an occasional animal in this group died without showing bacteremia. More deaths occurred at both X-ray doses among mice subjected to daily bleedings in the group given saline than among those merely observed for mortality data. Thus the course of events in the mice from which repeated blood samples were taken may not be strictly comparable to that among those not bled. Fig. 1 shows the results of blood cultures on representative animals from an experiment at 550 r.

Summary. Intravenous inoculation of mice with *Proteus* following moderate total body X-irradiation caused a marked increase in mortality and a much shorter median death time compared to control animals injected with saline. A severe bacteremia existed before death in these animals. Non-irradiated mice given the same inoculum of *Proteus* quickly reduced the number of bacteria in the blood with eventual complete clearing. No deaths occurred among these animals. Bacteremia caused by organisms common to the

intestinal tract frequently occurred in the mice which died following irradiation and saline injection.

1. Warren, S. L., and Whipple, G. H., *J. Exp. Med.*, 1923, v38, 713.
2. Chrom, S. A., *Acta Radiol.*, 1935, v16, 641.
3. Lawrence, J. H., and Tennant, R., *J. Exp. Med.*, 1937, v66, 667.
4. Furth, F. W., Coulter, M. P., and Howland, J. W., *Am. J. Path.*, 1952, v28, 171.
5. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science*, 1950, v111, 540.
6. ———, *J. Lab. Clin. Med.*, 1951, v38, 331.
7. Miller, C. P., Hammond, C. W., Tompkins, M., and Shorter, G., *J. Lab. Clin. Med.*, 1952, v39, 462.
8. Furth, F. W., Coulter, M. P., and Howland, J. W., *Am. J. Path.*, 1952, v28, 25.
9. Shechmeister, I. L., and Bond, V. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 77.
10. Shechmeister, I. L., Paulissen, L. J., and Fishman, M., *Fed. Proc.*, 1952, v11, 146.
11. Stuart, C. A., Personal Communication, 1952.
12. Gowen, J. W., and Zelle, M. R., *J. Infect. Dis.*, 1945, v77, 85.
13. Shechmeister, I. L., Bond, V. P., and Swift, M. N., *J. Immunol.*, 1952, v68, 87.

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Effects of the Hyperglycemic-Glycogenolytic Factor (HGF), Epinephrine and Insulin in Normal and Depancreatized Dogs.* (19715)

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During the course of experiments designed to study the action of certain insulin-free pancreatic extracts on the ketonemia and ketonuria of depancreatized dogs, it was noticed that the extracts had a marked hyperglycemic effect(1), probably because they contained the hyperglycemic-glycogenolytic factor (HGF). The extent of the hyperglycemia and its duration in different animals were extremely variable, however, even when the extracts were derived from the same batch of pancreas, were treated in the same manner and were injected under apparently identical conditions. Other experiments showed that the injection of epinephrine into depancreatized dogs with ketosis was also followed by a variable hyperglycemic response. The work described in this paper was undertaken to investigate the reasons for this variability.

Materials and methods. Well-fed mongrel dogs were depancreatized aseptically under sodium pentobarbital anesthesia. Following the operation, 100 ml of 0.9% saline, 100 ml of 10% glucose, 10 units of regular insulin (Lilly) and 30,000 of slow-acting penicillin were injected subcutaneously. This treatment was repeated on the first and, occasionally, on the second postoperative day. Penicillin injections were continued for 7 to 10 days. As soon as the animals started to eat, protamine zinc insulin (Lilly) was given instead of regular insulin. On the 7th postoperative day, the abdominal incision showed satisfactory healing and the stitches were removed. The dogs were kept in metabolism cages and fed the following daily ration: raw pancreas 100 g, sucrose 40 g, raw horse meat 500 to 700 g, depending upon the size of the animal. The urine was collected daily and the 24-hour glucose excretion was determined quantitatively; urinary ketone bodies were estimated according to an arbitrary scale (1+ to 4+) using Acetest§ tablets. The dogs were weighed once a week until the dietary and insulin requirements had been determined and

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§ Acetest Tablets were donated by the Ames Co., Elkhart, Ind.