determine qualitatively the presence of cycasin and MAM in fetal tissues, a quantitative recovery of the two compounds at various times after a single intragastric dose of cycasin was performed in one group of rats (Table I) and in the hamsters (Table III). The data for fetal rats shows that cycasin was present at 2 and 3 hours after its administration and absent thereafter, whereas the amount of MAM increased reaching a peak between 5 and 16 hours after which it slowly decreased to zero levels by 52 hours. This probably means that some of the cycasin was absorbed from the gastrointestinal tract without prior conversion to the aglycone and that it circulated for a brief period before it was excreted. The MAM, on the other hand, was found much longer in the fetuses. Since MAM is a highly unstable compound, it must be assumed that its prolonged demonstration in the fetus resulted from continued deglucosylation of cycasin by bacterial enzymes in the intestinal tract and subsequent absorption of the MAM thus produced. That the MAM remained in tissues for only a short period of time may be seen from the data in hamsters which received a large dose of MAM intravenously (Table III). Whereas a substantial percentage of the injected MAM could be found after 30 min, only a trace of MAM was left after 3 hours and none after 4 hours.

Summary. The passage of cycasin and MAM through the placenta and mammary gland has been investigated in rats during pregnancy and lactation, respectively. Both cycasin and MAM cross the placenta when cycasin is given orally to pregnant rats. Both compounds can also pass the mammary gland and are excreted with the milk during lactation provided that they are administered within 1 day before littering or during lactation. MAM has also been found in the fetuses after its intravenous injection into the pregnant golden hamster. The recovery of MAM from the fetuses of rats and hamsters supports a direct action of the carcinogen and teratogen on fetal tissues.

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Protection of Mice by Postirradiation Treatment with a Cell-Free Component of Spleen* (32673)

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The existence of a chemical entity of tissue origin which can be administered after radiation exposure and still protect against radiation injury was first suggested by Ellinger (1), who demonstrated that cell-free extracts of spleen caused a 10% reduction in mortality. In subsequent studies, the postirradia-

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ation mortality was unaltered (2) or was reduced as much as 40% (3) by administration of cell-free extracts of whole spleen. Katz and Ellinger (4), using ethyl alcohol fractionation procedures, obtained cell-free spleen extracts that reduced radiation mortality about 25%. This report will describe experimental conditions wherein a 94% reduction in radiation mortality was obtained by the postirradiation administration of a cell-free extract of splenic tissue. This highly protective fraction was obtained by gel filtration of Ellinger's splenic extract.

Materials and Methods. The initial extraction procedures for preparing cell-free spleen extracts were developed by Ellinger (1). Spleens were removed from 20 adult males obtained from our laboratory strain of albino mice. Spleens were frozen on dry-ice for at least 15 minutes and ground to a fine powder with a precooled mortar and pestle. The powdered spleens were leached in 15 ml of physiological saline solution (PSS) for 24 hours at 5°C, centrifuged at 1050*g* for 30 minutes at 5°C, and sterilized with an asbestos-cellulose filter (type ST, Hercules Filter Corporation). The filter pad was washed with sufficient PSS to bring the final volume up to 16 ml. This extract is similar to the crude extract by Ellinger, and will be so designated. Fifteen ml of Ellinger's extract was placed on a Sephadex G-200 column measuring 2.5 cm in diameter and 87 cm in length. The eluant used was PSS, and the flow-rate was 15–20 ml/hour. Fifteen-ml fractions were collected and the optical density was read at 280 mμ. The fractions were sterilized with a Millipore filter (pore diameter, 0.45 μ) and stored at —20°C until used. At no time was the temperature of the extract allowed to exceed 5°C.

Albino mice weighing 25±3 gm were irradiated ten at a time in a paper-covered stainless steel container with a Westinghouse Quadrocondex X-ray machine. Radiation factors were: 100 KVP; 20 mA; inherent filtration, hvl 0.77 mm Cu; target distance to surface where mice were standing, 50 cm; and dose rate, 41.5 r/min. Treatments with either PSS, Ellinger's extract or the fractions from the Sephadex column were started immediately after radiation exposure and consisted of five daily injections of 1 ml each. All injections were given subcutaneously in the area above the spleen. To minimize postirradiation infection, mice were housed five to a cage, and cages were cleaned and disinfected daily. Mortality results were recorded 20 days after X-ray exposure.

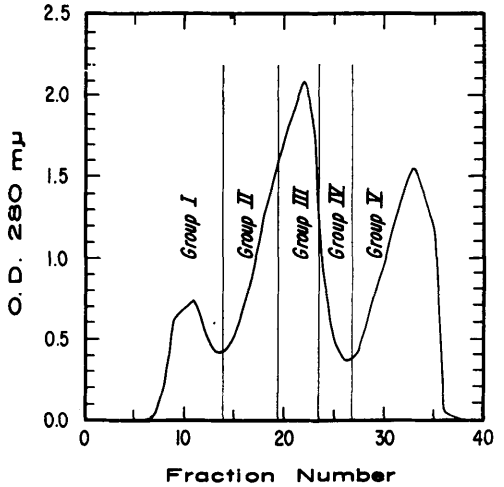

FIG. 1. Gel filtration of Ellinger's cell-free spleen extract with Sephadex G-200. Charted optical density was obtained by multiplying the optical density by the dilution necessary to lower the reading to less than 1.

Results. Figure 1 shows the optical density at 280 mμ of the fractions from Ellinger's crude spleen extract as they were collected from the Sephadex G-200 column. Under the prescribed conditions, gel filtration yielded a fraction which had three distinct protein peaks. Initially fractions were randomly selected for testing from all protein-rich eluants. For ease of presentation the fractions were divided into five general groups. The vertical lines on Fig. 1 are used to designate the fraction locations for each of these groups.

The results recorded on Table I are the cumulative results of some eight protection experiments. The results obtained with the crude spleen extracts are in accord with those of Ellinger (3), who reported that the splenic extracts increased survival by 30–40% after X-irradiation. The administration of fractions in Group I which were from the first peak did not alter the 100% mortality but caused a slight increase in survival time.

TABLE I. Protection of Mice against a 600r Total Body X-Ray Exposure by the Postirradiation Administration of Cell-Free Spleen Fractions.

Group	Treatment[a]	Average days survival[b]	Survival ratio	% Survival (20 days)
I	First peak fractions	7.3	0/12	0
II	First part of 2nd peak fractions	9.0	2/15	13
III	Top of 2nd peak fractions	19.3	44/47	94
IV	Last part of 2nd peak fractions	9.1	2/13	15
V	Third peak fractions	7.8	3/22	14
Control	PSS	6.9	0/66	0
Extract	Crude extract	12.3	11/27	40

[a] All mice received 5 daily 1-ml subcutaneous injections commencing immediately after radiation.

[b] The maximum survival time used in calculating average day of survival was 20 days, even though practically all mice that survived 2 weeks lived for several months.

Most of the protection against a lethal dose of radiation was associated with the second protein peak with only slight protection elicited by fractions from the third peak. When the protective second peak was divided into three parts, the protective factor was primarily confined to the three 15-ml fractions at the top of the second peak (Group III). Fractions from both the upward and downward slopes of the second peak induced limited protection. Only 3 of 47 mice receiving Group III fractions died following a 600r exposure which was lethal for 100% of the 66 control mice.

In addition to the protective factor, the fractions of the second protein peak with the highest optical density also contained most of the hemoglobin. Consequently, it is assumed that the protective chemical has a molecular weight of approximately 70,000. Group III splenic extracts have been either frozen and thawed twice, stored at 5°C for 5 days, or frozen for 6 months at —20°C without losing protective activity.

The concentration of proteins in the fractions which protected greater than 90% of mice was approximately one-sixth that of the crude splenic extract which protected only 40% of the mice. Furthermore, each mouse injected with crude extract received material from 6.25 spleens, and those receiving the highly protective fractions received the material prepared from 2.22 mouse spleens. Consequently, it is assumed that gel filtration results in either the release of the protective entity or removal of inhibitors.

Table II shows the cumulative results of experiments in which Group III extracts were used to protect against different doses of X-irradiation. Even though survival times were almost doubled and some mice receiving the splenic material survived 900 and 1200r doses of total body X-irradiation, the degree of protection induced by the postirradiation treatment was not as great as with 600r.

Discussion. X-irradiated mice have been protected by postirradiation splenic implantation (5), administration of homogenates of normal mouse spleen (6), and the administration of subcellular fractions of splenic tissue (7). With each of these treatments the possibility exists that the protection was associated with the implantation of living cells, even though this would have been mini-

TABLE II. Protection of Mice with Cell-Free Splenic Extracts Administered Following Exposure to Different Doses of Total Body X-Irradiation.

Group	Average days survival	Survival ratio	% Survival (20 days)
1200r, Rx[a]	7.7	1/9	11
1200r, PSS	4.9	0/9	0
900r, Rx	13.5	4/10	40
900r, PSS	5.0	0/9	0
600r, Rx	19.3	44/47	94
600r, PSS	6.9	0/66	0

[a] Rx mice were given 5 daily 1-ml injections of Group-III fractions from mouse spleen.

mal with the protective nuclei fractions used by Cole *et al.* They used differential centrifugation in sucrose to obtain their protective nuclei fractions. The possibility that cells could be implanted in the present study is eliminated by the fact that all protective extracts were sterilized by both Seitz and Millipore filtration.

Cole and Ellis (8) reported that the protective activity of nuclei fractions was destroyed by DNase and trypsin. The exact chemical nature of the protective fraction in this study is unknown.

Summary. Fractions of Ellinger's cell-free splenic extract administered following a lethal X-ray exposure resulted in 94% survival. This increased survival is greater than has previously been reported with any cell-free preparations given after radiation exposure. The protective agent was found in the second protein peak obtained by gel filtration with Sephadex G-200. The molecular weight of the protective fraction as determined by gel filtration was approximately 70,000. The relative degree of protection induced by this cell-free factor decreased with increasing X-ray doses.

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Specificity of the Inhibitory Effect of "Uremic" Serum on *p*-Aminohippurate Transport* (32674)

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Several recent reports have demonstrated that addition of uremic serum from human patients with diseased kidneys or from bilaterally nephrectomized rats inhibits the *in vitro* accumulation of *p*-aminohippurate (PAH) by slices of rat renal cortex or isolated fragments of rabbit renal cortex (1–3). These data raised two important questions that required answering before a complete and thorough interpretation of these results could be made. First, preliminary work in our laboratory demonstrated that if rats were fasted for 24 hours prior to sacrifice and preparation of *in vitro* slices, the ability of these slices to accumulate PAH was significantly impaired. Inasmuch as bilaterally nephrectomized rats would not be expected to eat as much as normal rats, could the effect of nephrectomized serum on PAH accumulation merely be due to the fasting state of the donor animals rather than to the uremia produced by nephrectomy? Second, is the effect of nephrectomized serum on PAH accumulation specific for organic anion transport or is this a general depressant effect upon other functions of the *in vitro* slices as well? An attempt was made to answer these questions by measuring the simultaneous uptake of PAH and the organic cation *N*-methylnicotinamide (NMN). Experiments were first conducted on renal cortical slices taken from fasted and nonfasted rats. Subsequent experiments were then conducted by using renal cortical slices from nonfasted rats and determining the effect of addition of serum from nephrectomized or sham-nephrectomized rats on the uptake of PAH and NMN by these slices.

Methods. Male Sprague-Dawley Rats,

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