The manner in which protein-depletion increases susceptibility to radiation is not known. Several methods by which radiation may cause death have been postulated and protein-depletion affects most of these mechanisms. Death following radiation may be due to infection subsequent to the break-down of the normal immunological processes. It is well known that protein-depletion interferes with immunological responses and this in combination with the effects of radiation on the epithelial barriers, particularly the intestinal epithelium, may account for the increased susceptibility. Also it may be more difficult for the depleted animals to recover from the leukopenia of radiation so that the phagocytic barrier against infection is lowered. There is much evidence that the degree and duration of leukopenia following radiation have great influence on mortality.[27]

Since it is known that protein-depletion and radiation both interfere with enzyme production or action, it may be that there is an additive effect that interferes with enzyme activity to account for the increased radiation susceptibility.

It may be that death is due to anemia following bone marrow damage. That this mechanism accounts for the increased susceptibility does not seem likely for protein-depletion *per se* does not cause a marked lowering in the erythrocyte or hemoglobin levels. However it may interfere with the recovery of the bone marrow and with the production of both erythrocytes and leukocytes.

It seems possible that the increased susceptibility to radiation observed in these protein-depleted animals might be due to their poor immunological and leukocytic responses or to their decreased production of some essential enzyme. This problem is being investigated further.

Conclusions. 1. Protein-depletion in young adult male rats, as produced by a weight loss of 25% on a protein-poor diet, materially increases susceptibility to total body irradiation. 2. Age or weight in normally nourished adult rats, in the 150 to 280 g weight range, have little effect on susceptibility to radiation.

The author wishes to express his appreciation for advice and assistance to the following men: P. R. Cannon, R. W. Wissler, and E. P. Benditt, Department of Pathology, University of Chicago; A. M. Brues, Biology Division, Argonne National Laboratory, Chicago; L. J. Savage, Department of Mathematics, University of Chicago; and H. D. Landahl, Committee on Mathematical Biology, University of Chicago.

The animals used in this experiment were irradiated through the collaboration of the Biology Division, Argonne National Laboratory Chicago.

[27] Brues, A. M., and Rietz, L., ANL-4227, **183**, 1948.

Received October 25, 1949. P.S.E.B.M., 1949, **72**.

An Autohemolytic Agent in Fetal Liver Extracts.* (17479)

DAVID B. TYLER

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.

In the course of studies dealing with enzymatic activity during fetal and extrauterine development it was noted that extracts of fetal liver lysed the red cells of the same species. It was of interest to determine the nature of this phenomenon and to compare the activity of adult and fetal extracts.

Methods. Thirty experiments were performed, mostly on pregnant guinea pigs and their fetuses. A few experiments were made with pregnant rats or newborn rats and their mothers. The pregnant animals were killed by decapitation and the maternal blood was collected in oxalated tubes. The fetuses were then quickly removed and decapitated and

* This investigation was supported in part by a grant from the National Cancer Institute, U. S. Public Health Service.

the blood collected in separate oxalated tubes. The fetal and maternal livers, spleens, kidneys, hearts and lungs were perfused *in situ* to wash out as much blood as possible and then removed and frozen on dry ice. When all the tissues were removed and frozen they were weighed, then ground in a porcelain mortar and 0.9% NaCl was added to obtain suspensions varying from 5 to 30%. These suspensions were centrifuged and the supernatant fluid was used in subsequent tests.

In the experiments reported here the activity of each extract was tested on both fetal and maternal red cells. In several runs a suspension of red cells was used from a species other than the one from which the tissue extracts were prepared. In all instances, the cells were washed three times with 10 to 12 volumes of physiological saline and used in the test as a 1% suspension.

The supernatant fluid of the tissue suspension was diluted by pipetting into several 12 x 75 mm test tubes 1.0, 0.5, 0.25 and 0.10 ml of the extract and making them up to 1.0 ml with 0.9% saline. Several tubes containing 1.0 ml saline were prepared to serve as controls. One ml of the red cell suspension was then added to these tubes. Since some fetal liver extracts, despite *in situ* perfusion, possessed interfering color it was necessary to set up a series of tubes containing various dilutions of the extract together with 1.0 ml of saline instead of the red cell suspensions. These tubes served as color controls. The tubes were placed in an incubator (38°C) and at one-half hour intervals they were shaken. After 2½ to 4 hours incubation they were removed and centrifuged at 2,000 RPM for 5 minutes. The degree of hemolysis was determined visually by the amount of color in the supernatant liquid (corrected for by the "color controls") and by the amount of sediment. The usual grades, 4, indicating complete hemolysis, to 0, for none, were employed.

Results. As illustrated in Table I, extracts of fetal guinea pig liver (crown-rump size from 6 to 9 cm) lysed *in vitro,* and within 4 hours, suspensions of red cells from the blood of the same fetus, as well as from that of the mother. They also exhibited this lytic action on red cells from the male of the same species, as well as against a red cell suspension of rat blood. This activity could be demonstrated in dilutions equivalent to 0.5% of the original suspensions. Extracts of maternal guinea pig liver exhibited detectable activity only in relatively high concentrations and, in the experiments reported here, failed to lyse within the 4 hour period the red cells of the rat. Extracts of other fetal tissues (Table II), with the possible exception of the spleen, did not show as high activity as that found for liver, nor were the fetal-maternal differences as consistent as those found for liver. Extracts of fetal guinea pig spleen, in 2 experiments, and fetal kidney, in one experiment, possessed greater lytic activity than the corresponding maternal extracts. In the rat, fetal liver and spleen possessed greater activity than the maternal organs. Extracts of adult rat lung and adult guinea pig lung appeared to produce more lysis than the corresponding fetal organs. However, in these experiments very little lysis was produced after 4 hours.

Boiling the extract of fetal guinea pig liver for one minute completely destroyed the activity (Table I.). The precipitate of such a heated extract caused a small amount of hemolysis at the end of 4 hours, indicating that a heat-stable substance was carried down by the precipitate. Heating for one hour at 56°C destroyed about 75% of the activity. The addition of fresh serum to such a heat-inactivated extract did not restore the activity. Apparently complement, in the classical sense, is not involved in the hemolysis. Allowing the extracts to stand for one hour with heated (to 56°C) or fresh serum before the red cells are added reduces the amount of hemolysis. This may mean that an anti-hemolytic factor that is not a protein is present in the serum.

If the complete system (extracts plus red cell suspension) is allowed to stand overnight, a marked increase in hemolysis is observed and, in some experiments with liver extracts, the fetal-maternal differences are obscured.

TABLE I.
Hemolytic Action of Saline Extracts of Fetal and Maternal Guinea Pig Livers.

	Dilution (see text)			
	15.0	7.5	3.8	1.5
A. Against maternal red cells				
1. Unheated				
Fetal	++++	++++	++	±
Maternal	+	±	0	0
2. Boiled 1 min.				
Fetal	0	0	0	0
Maternal	0	0	0	0
3. Ppt. of boiled extr.				
Fetal	+	±	0	0
Maternal	0	0	0	0
4. Heated to 56°C 1 hr				
Fetal	++	++	0	0
Maternal	0	0	0	0
5. Effect of 0.5 cc serum (See text)				
Fetal	+++	++	±	0
Maternal	0	0	0	0
	20.0	10.0	5.0	2.5
B. Against fetal red cells				
Fetal	++++	+++	++	0
Maternal	±	0	0	0
	15.0	7.5	3.8	1.5
C. Against rat red cells				
Fetal	++++	+++	++	0
Maternal	0	0	0	0

If the extracts alone are allowed to stand for 12 hours and then tested, a marked increase in their ability to produce lysis is found. On the other hand, the addition of the sodium salt of monoiodoacetic acid to the extracts in a final concentration of 0.003 M inhibits the increase of activity due to standing.

Discussion. The results indicate that saline extracts of fetal guinea pig liver possess greater lytic activity than similarly prepared adult (maternal) extracts. The activity is due to both heat-labile and heat-resistant factors but the heat-sensitive agent accounts for the greater part of the lysis under the conditions of these experiments. At present, we cannot account for the fetal-maternal difference reported here. There is, however, a considerable body of literature that may give us insight into the probable nature of some of the lytic agents or systems involved.

There is substantial support for the idea that at least 3 hemolytic systems involving lysins exist *in vivo:* (1) It is well known that the spleen plays an important role in preparing red cells for destruction. Rous,[1] Krumbhaar,[2] Dameshek,[3,4] and Ponder[5] have reviewed the evidence bearing on this. It has been suggested that the agent responsible for this action of the spleen is produced by enzymatic action and the resulting substance has been identified as lysolecithin.[6,7,8] This

[1] Rous, P., *Physiol. Rev.*, 1923, **3**, 75.

[2] Krumbhaar, E. B., *Physiol. Rev.*, 1926, **6**, 160.

[3] Dameshek, W., *New England Med. J.*, 1941, **224**, 727.

[4] Dameshek, W., *New England Med. J.*, 1942, **226**, 339.

[5] Ponder, E., Hemolysis and Related Phenomena, Grune and Stratton, 1948.

[6] Bergenhem, B., and Fahraeus, R., *Z. Ges. Exp. Med.*, 1936, **97**, 555.

TABLE II.
Hemolytic Action of Saline Extracts of Other Tissues Using Maternal Red Cell Suspensions.

	Dilution (see text)			
	15.0	7.5	3.8	1.5
A. Guinea pig				
1. Spleen				
Fetal	++	+	0	0
Maternal	+	+	0	0
2. Kidney				
Fetal	+	±	0	0
Maternal	±	0	0	0
3. Lung				
Fetal	0	0	0	0
Maternal	++	++	+	+
B. Rat				
1. Liver				
Fetal	++++	+	±	0
Maternal	++	0	0	0
2. Spleen				
Fetal	++++	++++	+++	+
Maternal	±	±	0	0
3. Lung (Homogenized in blendor)				
Fetal	++	++	+	±
Maternal	+++	+++	++	+

system is heat-sensitive and is inactivated at 56°C. (2) That lysins can be obtained from tissues other than the spleen has been shown by Metchnikoff,[9] Weil,[10] Maegraith, Findlay and Martin,[11] and Ponder.[12] Weil found that kidney and liver extracts contained lysins and that their activity was inhibited by serum. He also pointed out that the lytic activity of some extracts may be derived from residual blood in the tissue. In the experiments reported here it was extremely difficult to obtain blood-free saline extracts of fetal liver. However, it should be pointed out that the lytic action of fetal liver was equally as potent against the red cells of the same fetus as against the maternal or rat blood cells. Maegraith, Findlay and Martin[11] have recently described a heat-sensitive agent found in a variety of human and animal tissues. This agent produces lysis after 24 hours of incubation at 37°C. Their lysin is inactivated at 80°C in 5 minutes and they report it to be species-specific. Ponder[12] has been able to confirm in part the observations of Maegraith, *et al.*, but he finds that extracts he prepared are not species-specific. Weil[10] did not determine whether his extracts lysed the red cells of other species. The heat-sensitivity and lack of species-specificity suggest that the predominant factor in the fetal liver is similar, as far as those two characteristics are concerned, to the agent found by Ponder,[12] but differ as to the time required to produce lysis. This may be due to a higher concentration of the factor in fetal liver. The results also suggest that it may be lysolecithin, or some closely related substance. The inhibitory action of iodoacetate in preventing the accumulation of such lytic agents might be accounted for by its activity on the enzyme responsible for the production of the lysolecithin.

7 Singer, K., *Am. J. Med. Sc.*, 1940, **199**, 466.

8 Singer, K., *J. Clin. Inv.*, 1941, **20**, 153.

9 Metchnikoff, cited by Weil.[10]

10 Weil, R., *J. Med. Research*, 1907, **16**, 287.

11 Maegraith, B. G., Martin, N. H., and Findlay, G. M., *Brit. J. Exp. Path.*, 1943, **24**, 58.

12 Ponder, E., *J. Gen. Physiol.*, 1944, **27**, 483.

Although heat-labile factors are responsible for the greater portion of the activity reported in these experiments a small amount is found to be due to heat-resistant substances. This brings up the question of their probable nature.

(3) In addition to the two hemolytic systems briefly described above there is evidence for the existence of a third system. Freeman and Johnson[13] and Loewy, *et al.*[14] have shown that an increase in soaps in chyle, particularly during fat absorption after a meal high in fats, can be large enough to bring about a degree of hemolysis on entering the blood. The fetal guinea pig liver, at the stages studied, has a very high fat content[15] and it is conceivable that as a result there is a sufficient increase in the soap content to account for the heat-stable hemolytic agent.

The fact that serum heated, as well as fresh, inhibits the activity of the lytic agents points to the probability that this may be part of an *in vivo* counteracting mechanism.

Bearing on the general phenomena reported here is the recent observation of Gross[16,17] to the effect that saline extracts from spontaneous mouse mammary carcinomas and human cancer frequently hemolyse red cells *in vitro*. With the former preparation hemolysis was observed after 2 to 3 hours incubation whereas with the latter it became evident only after 24 hours. Weil[10] also reported a similar study in which he observed that non-necrotic tumors behaved like normal tissue while necrotic tissue apparently produced a different kind of a lytic agent. Both Weil and Gross offer their findings as the basis for an explanation of the anemias which frequently occur during malignancies.

It should be noted that in respect to time of incubation needed to produce lysis fetal liver extracts resemble extracts of spontaneous mouse mammary carcinoma.

Summary. (1) A saline extract of the liver of fetal guinea pig possesses greater hemolytic activity than a similarly prepared extract of maternal liver, as determined after 4 hours incubation at 38°C.

(2) The activity, for the most part, is due to a heat-sensitive agent and, to a lesser extent, to a heat-resistant substance.

(3) The lytic action of such extracts is not species-specific. It will lyse red cells from the same fetus, as well as those from the mother or from another species.

13 Freeman, L. W., and Johnson, V., *Am. J. Physiol.*, 1940, **130**, 723.

14 Loewy, A., Freeman, L. W., Marchello, A., and Johnson, V., *Am. J. Physiol.*, 1943, **138**, 320.

15 Imrie, C. G., and Graham, S. G., *J. Biol. Chem.*, 1920, **44**, 243.

16 Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 292.

17 Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 656.

Received Sept. 1, 1949. P.S.E.B.M., 1949, **72**.

Amino Acid Composition of Morphological Fractions of Rat Livers and Induced Liver Tumors.* (17480)

B. S. SCHWEIGERT AND BARBARA T. GUTHNECK, J. M. PRICE,† J. A. MILLER, and E. C. MILLER

From the American Meat Institute Foundation and Department of Biochemistry, University of Chicago, McArdle Memorial Laboratory, University of Wisconsin.

In previous studies,[1,2] comparisons were made of the chemical compositions of the nuclear, large granule (mitochondria), small granule (microsome), and supernatant fluid fractions of the livers from normal rats, the livers of rats fed the hepatic carcinogen 4-dimethylaminoazobenzene, and the liver tumors induced by this dye. Ingestion of the dye resulted in altered levels of protein, nucleic acids, and riboflavin in certain fractions. In particular, increased levels of protein in the nuclear fraction and decreased