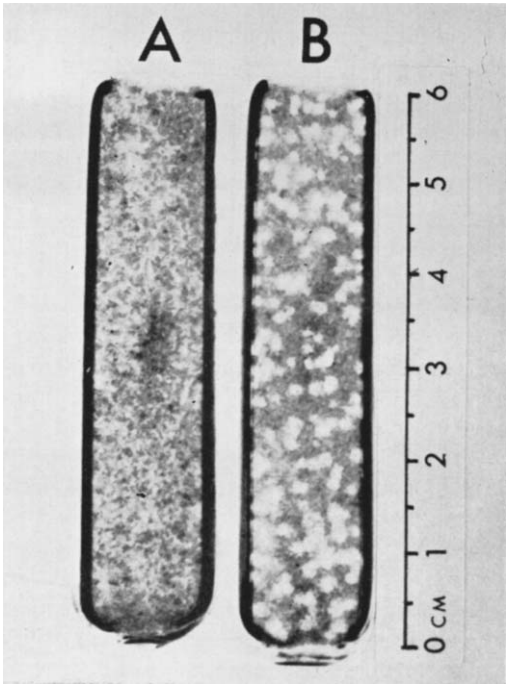


FIG. 3. Control culture and plaques obtained with herpes simplex virus grown 50 hr on BSC cell cultures in Leighton tubes. Carbolfuchsin (Ziehl-Neelsen stain).

hours. In our studies, the methyl cellulose was essential for circumscribing the viral growth and providing sharply outlined plaques, and thus afforded a distinct advantage over similar procedures in which methyl cellulose was not employed.

Summary. Methods have been described for quantitative plaque assay of herpes simplex virus either in bottle culture or in Leighton tubes. The overlay employed was devoid of agar, peptones and serum, which are viewed as undesirable constituents. Use of agar overlay results in production of plaques from only a small fraction of the potential plaque-forming population. The inhibition of plaque formation by agar can be reversed by addition of protamine sulfate at a level of approximately 800 μg/ml. Possible uncertainties associated with the use of mixtures of agar and protamine can be avoided by substitution of methyl cellulose with which a further 10-fold increase in sensitivity of the assay was obtained.

While this manuscript was in preparation, a paper was published in *Virology*, 1963, v19, 40, by I. T. Schulze and R. W. Schlesinger describing a methyl cellulose overlay for plaquing of arthropod-borne viruses.

1. Dulbecco, R., *Proc. Nat. Acad. Sci.*, 1952, v38, 747.
2. Kaplan, A. S., *Virology*, 1957, v4, 435.
3. Farnham, A. E., *Virology*, 1958, v6, 317.
4. Russell, W. C., *Nature*, 1962, v195, 1028.
5. Liebhaber, H., Takemoto, K. K., *Virology*, 1961, v14, 502.
6. Tytell, A. A., Torop, H. A., McCarthy, F. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 723.
7. Tytell, A. A., Torop, H. A., McCarthy, F. J., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 916.
8. Kaverin, N. V., *Problems in Virology*, 1962, v6, 805.

Received April 8, 1963. P.S.E.B.M., 1963, v113.

Studies on Fibrinogen Turnover Before and After Whole Body X-Irradiation in the Rat.* (28363)

GARY S. SHABER AND LEON L. MILLER
Department of Radiation Biology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Various authors have reported the normal biological half-life of fibrinogen in several species to be between 1 and 5 days(3,5,7,8, 11,12). The biological half-life of fibrinogen following whole-body irradiation has not been reported. The plasma level of fibrinogen in dogs rises above normal concentrations to a peak 3 to 5 days after X-irradiation(4).

Fibrinogen survival in animals shows an

* These studies were performed under contract with U. S. Atomic Energy Commission at Univ. of Rochester Atomic Energy Project, Rochester, N. Y.

exponential disappearance curve, with a rapid initial period of turnover and then a slower phase(1,5,12). This change in rate of turnover has been attributed to the fact that the labeled fibrinogen passes out of the vascular space and comes to equilibrium with the extravascular pool of fibrinogen. Equilibrium with the large amount of fibrinogen present extravascularly is thought to be reached in 2 to 4 days(3,5).

The intent of this study was to determine the turnover of fibrinogen in the rat following whole-body irradiation. Because of the reported biphasic nature of the turnover of fibrinogen, we thought it of interest to expose animals to radiation at times corresponding to both segments of the turnover curve. Accordingly, our study was divided into 3 experiments. In the first, a group of rats was irradiated 6 days after injection of I^{131}-fibrinogen. A second group was irradiated immediately after injection of labeled fibrinogen. In a third group, animals were irradiated immediately after injection of labeled fibrinogen, then serially sacrificed so that a time comparison could be made between whole body and plasma radioactivity.

Material and methods. Rat fibrinogen was prepared according to the method of Mihalyi (10). It proved repeatedly to be 90% clottable by Kjeldahl analysis. The fibrinogen† was labeled with I^{131} using I^{131} monochloride (2). To eliminate denatured fibrinogen, the freshly prepared labeled fibrinogen was screened *in vivo*(9). Eighteen hours after intravenous injection through a femoral catheter into 300 g Sprague-Dawley male rats, plasma was harvested from the blood of these rats and diluted with physiological saline so that 1 ml contained about 100 μc of radioactivity.

The experimental Wistar strain rats, all males, were housed in separate metabolic cages. To minimize localization of the metabolized I^{131} in the thyroid, the drinking water consisted of 0.0025 M KI from one day prior to injection of labeled plasma until the end of experiment. Each rat received *via* the femoral vein 1 ml of plasma containing I^{131}-labeled fibrinogen. This plasma will hereafter be referred to as I^{131}-fibrinogen. Immediately, and daily thereafter, total body radioactivity was measured in a whole body counter. Background counts were taken for 5 minutes and the rats were counted for one minute. Twenty-four hour urine volumes were recorded daily for each rat and a 1 ml aliquot counted for 2 minutes in a well-type scintillation counter. The counting error averaged 2.8% of the total counts and was never higher than 6.5%.

The reference standard used in whole-body radioactivity determinations was an amount of iodine-131 equal to that injected into the test animals. This was placed in a beaker with approximately the same physical geometry as the animals and its radioactivity was measured before and after the animals were counted each day. From these data a mean value was obtained, and all animal counts were compared to this standard after correction for physical decay and adjustment to activity at time zero. A similar reference standard consisting of a smaller aliquot of dose was used for the urine specimens.

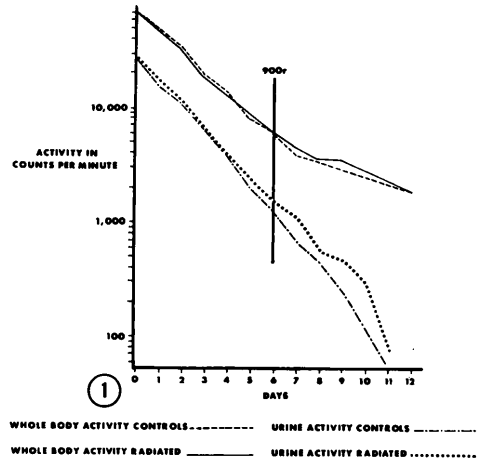
Irradiated animals received 900 roentgens of X-ray midline air dose to the whole body from an overhead KVP machine operating at 15 ma with a target skin distance of 25 inches and with an approximate dose rate of 18 r per minute. Although all rats were allowed access to food and water after irradiation, the irradiated rats ate very little food, and appeared somewhat dehydrated as estimated by decreased skin turgor. There was no gross evidence of hemorrhage.

Experiment 1. Sixteen rats weighing 300 g were injected with I^{131}-fibrinogen. After 6 days, 8 of the 16 rats received 900 r; the remaining 8 rats served as controls.

Experiment 2. Eight rats weighing 300 g were used. Four rats were irradiated immediately after injection of I^{131}-fibrinogen. Four rats injected with I^{131}-fibrinogen at the same time served as non-irradiated control.

Experiment 3. Thirty rats weighing 200 g were divided into 2 groups of 15 each and injected with I^{131}-fibrinogen. At the outset one group received 900 r; the second group

† We are indebted to Dr. William F. Bale and Miss Letitia E. Mutschler for generously preparing and supplying I^{131}-fibrinogen.



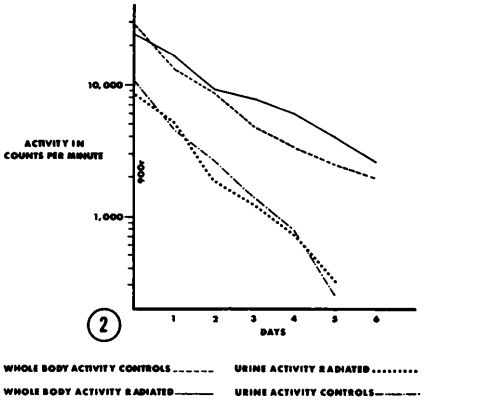


FIG. 1. Turnover of fibrinogen following delayed whole body irradiation.

FIG. 2. Turnover of fibrinogen following immediate whole body irradiation.

served as control. Three rats from both experimental and control groups, chosen using a random table, were sacrificed daily for 5 days. Measurements were made of radioactivity in 1 cc of plasma from all rats. From one rat in each group of 3 radioactivity was measured in the thyroid, the injection site, and in a piece of thigh of similar weight from the leg opposite the injection site. In a similar study not reported here in detail the small intestine (plus contents) of irradiated and control rats were compared for radioactivity.

Results. Exp. 1. A semilog plot of the mean daily whole-body counts *vs* time was made for each group of rats and the results are presented in Fig. 1. Also plotted is the mean radioactivity in urine of control and experimental groups. For each rat the total radioactivity excreted in urine during the entire experiment was used to plot the initial urine value. The amount excreted each day was subtracted consecutively from this total. Thus, urine radioactivity as plotted decreases progressively until, after the last day, it is zero. This was done so that the plot would correspond graphically to the whole-body turnover curve.

From Fig. 1 the apparent biological half-life prior to radiation is 1.5 days for both control and experimental animals. Following the seventh day, the apparent biological half-life appears to change from 1.5 days to 2.7 days for the control animals. Following radiation on day 6, the biological half-life changes from 1.5 to 3.2 days for the irradiated animals. The increase observed in both control and irradiated animals proved to be statistically significant. The one-half day difference in biological half-life between the irradiated and control animals following day 7 is not significant statistically. Thus, on the basis of whole body radioactivity, both control and experimental groups had similar total turnover of fibrinogen during this period.

The gross pattern of urinary excretion in this and subsequent experiments corresponded to the changes in whole body activity. However, no quantitative estimate of the biological half-life has been made on the basis of the urinary data because of unpredictable variation in bladder emptying, and because of occasional losses occurring during handling of the rats.

Exp. 2. The results of this experiment as depicted in Fig. 2 reveal no apparent difference in whole-body I^{131} activity when irradiation was given immediately after injection of the labeled fibrinogen. Here again I^{131} loss in urine closely parallels whole-body activity.

Exp. 3. For each rat a comparison was made between the activity present in 1 ml of plasma and whole-body counts at time of sacrifice. The plasma was counted in a well-type scintillation counter which, because of its geometry, is much more sensitive than the whole body counter. Because of this, the

TABLE I. Comparison of Whole-Body Activity to Plasma Activity: Rats Irradiated Day 1. Arbitrary ratio: Plasma activity/whole-body activity.

	Day				
	1	2	3	4	5
Controls avg ± S.E.	22.0 ± 3.5	23.1 ± 1.8	23.5 ± 2.0	18.9 ± 1.7	19.7 ± 2.2
Irradiated avg ± S.E.	18.1 ± 3.5	13.4 ± 1.8	12.5 ± 2.0	6.4 ± 1.7	5.7 ± 2.2
$t_{.05}$ test	0	+	+	+	+

0 = Not significant + = Significant.

ratios of whole-body activity to plasma activity are arbitrary relative values. The mean values of these ratios for both groups are shown in Table I. The daily values of the controls are almost constant while the ratios of activities of the irradiated animals decrease markedly. The irradiated group of animals had lower plasma activity in relation to whole-body counts than did the control group for every one of the 5 days. However, this difference was statistically significant only from the second day on.

Assays of the thyroid, injection site, and thigh, done at the time the animals were sacrificed, showed no significant difference in radioactivity per gram tissue present in these sites in the same animal. The small intestine plus contents of the irradiated rats, compared with the normal, had a lower radioactivity relative to the whole-body activity. The muscle and thyroid of the irradiated animals showed slightly higher radioaction activity (relative to the whole-body activity) than did the muscle and thyroid of the controls. A more precise localization of radioactivity in the tissues of irradiated I^{131} fibrinogen injected rats deserves further study.

Discussion. The most significant finding in these experiments is the accelerated disappearance in irradiated animals of labeled fibrinogen from the vascular space. This effect was seen early and became statistically significant on the second day. Gitlin *et al.*, have presented evidence that under normal conditions fibrinogen enters the lymphatics, interstitial spaces, and connective tissues of the human body(6). In Exp. 3 radioactivity was not found localized in the small intestine but was present to a slightly higher extent in the skeletal muscle and thyroid of the irradiated animals. It may be tentatively presumed that I^{131} fibrinogen was more broadly distributed throughout the extravascular compartment of the irradiated rats.

There was a slowing of the (normal) turnover rate after about 6 days in all normal control animals from an initial half-life of 1.5 days to 2.7 days. The very early change reported to occur in the first 24 hours after injection of the labeled protein by most authors was not seen here. From observations made in other species one may presume that the time required for the injected fibrinogen to become homogeneously distributed in the extravascular fibrinogen pool in the rat is significantly shorter than 24 hours, at which time our first post-injection measurements of plasma I^{131} were made. Also, because *in vivo* screened I^{131} fibrinogen was used in our experiments, any apparent shortening of turnover time referable to the presence of denatured I^{131} fibrinogen would have been minimized. When the fibrinogen turnover is examined by means of whole-body counts as in this study, any process that enhanced removal of fibrinogen from the vascular space without its being excreted would not appear as an apparent increase in whole-body turnover rate, although it would be reflected in an apparent decrease in the half-life of plasma fibrinogen as observed in Exp. 3.

Summary. 1. The biological half-life for *in vivo* screened I^{131} labeled rat fibrinogen injected intravenously in normal rats was found to be 1.5 days. After 6-7 days the apparent biological half-life changed to 2.7 days. 2. The apparent whole-body turnover of I^{131} fibrinogen after whole-body X-irradiation was the same for both irradiated and control rats. However, the data on plasma I^{131} fibrinogen indicate that fibrinogen leaves the vascular space at an enhanced rate after whole-body irradiation.

1. Adelson, E., Rheingold, J. J., Parker, O.,

Buenaventura, A., Crosby, W. H., *Blood,* 1961, v17, 267.

2. Helmkamp, R. W., Goodland, R. L., Bale, W. F., Spar, I. L., Mutschler, L. E., *Cancer Res.,* 1960, v20, 1495.

3. Christensen, L. Korsgaard, *Acta Med. Scand.,* 1958, v162, 407.

4. Colgan, J., Gates, E., Miller, L. L., *Serum and Urinary Fibrinolytic Activity Related to the Hemorrhagic Diathesis in Irradiated Dogs,* Univ. of Rochester Report No. 210, 1952.

5. Gitlin, D., Borges, W. H., *Blood,* 1952, v8, 679.

6. Gitlin, D., Landing, B. H., Whipple, A., *J. Exp. Med.,* 1953, v97, 163.

7. Hammond, J. D. S., Verel, D., *Brit. J. Hemat.,* 1959, v5, 431.

8. Madden, R. E., Gould, R. G., *J. Biol. Chem.,* 1952, v196, 641.

9. McFarlane, A. S., *Biochem. J.,* 1956, v62, 135.

10. Mihalyi, E., *Acta Chem. Scand.,* 1950, v4, 317.

11. Stefani, M., *Am. J. Med.,* 1953, v14, 64.

12. Volwiler, W., Goldsworthy, P. D., MacMartin, M. P., Wood, P. A., Mackay, I. R., Fremont-Smith, K., *J. Clin. Invest.,* 1955, v34, 1126.

Received September 14, 1962. P.S.E.B.M., 1963, v113.

Interlipid Relationships in Clinical Atherosclerosis.* (28364)

FREDERICK J. MOORE, JESSIE MARMORSTON AND OLIVER T. KUZMA

Departments of Public Health and Medicine, University of Southern California School of Medicine, Los Angeles County Hospital and Cedars of Lebanon Hospital, Los Angeles

In view of the large amount of evidence that cholesterol plays an important role in atherosclerosis(1), it is surprising that serum cholesterol levels have little predictive value for the occurrence of myocardial infarction or other complications of coronary atherosclerosis(2). Also, it is surprising that the average cholesterol level of "atherosclerotic" persons is only about 10% higher than "normals," while individuals in either group vary over a range of 300%(3). Lacking, therefore, evidence clearly tying the absolute level of serum cholesterol to clinical atherosclerosis, we have considered the possibility that there might instead be some significant abnormality in its relative level.

This approach is suggested by the report of Peters and Man(4) that there is a great constancy in the relations between cholesterol and the phospholipids in a wide variety of ages and clinical states. Somewhat later, Popjak(5) reexamined these and other data, and in addition data from other species, and found that the curve relating phospholipids (P) to cholesterol (C) became linear when plotted logarithmically. He, therefore, fitted the equation $\text{Log } P = a + b \text{ Log } C$ by the method of least squares and found the constants essentially identical for all age groups, all clinical states and all animal species examined. He concluded that the mathematical relationship between serum cholesterol and phospholipids was a biological constant. Any significant disturbance in this fundamental relationship should, therefore, have substantial implications.

Neither Peters and Man nor Popjak examined data from patients with myocardial infarction or cerebral thrombosis, and we have accordingly subjected our data on such patients, as well as healthy and chronically ill control groups, to the statistical procedures used by Popjak. In confirmation of the prior reports, we find that the 2 control groups have essentially identical relationships between cholesterol and phospholipids, and that these relationships are not affected by age. However, the 2 atherosclerotic groups, while being essentially identical themselves, both differ substantially from the controls. This indicates that there is indeed a distinct abnormality in the serum lipids of patients with atherosclerosis and that this abnormality occurs in a relationship that is otherwise constant over all ages, numerous other diseases and several other animal species.

* Supported by grants from Albert and Mary Lasker Foundation, Bella and André Meyer Foundation, and from Nat. Heart Inst., U.S.P.H.S. Laboratory determinations were under the direction of Drs. Ross Jacobs, John W. Mehl (Dept. of Biochemistry), and Sara M. Myers.