4. Choppin, P. W., Tamm, I., J. Exp. Med., 1960, v112, 895.
5. Adams, J., Jr., Imagawa, D. T., Proc. Soc. Exp. Biol. and Med., 1957, v96, 240.
6. Hopps, H. E., Bernheim, B. C., Nisalak, A., Tjio, J. H., Smadel, J. E., J. Immunol., 1963, v91, 416.
7. Tamm, I., Horsfall, F. L., Jr., J. Exp. Med., 1952, v95, 71.
8. Aminoff, D., Biochem. J., 1961, v81, 384.
9. Henle, G., Girardi, A., Henle, W., J. Exp. Med., 1955, v101, 25.
10. Takaba, Y., Kinuwaki, Y., Hayashi, H., Proc. Soc. Exp. Biol. and Med., 1964, v115, 906.
11. Okada, Y., Yamada, K., Tadokoro, J., Virology, 1964, v22, 397.

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## Effects of Trypsin on Ultraviolet Irradiated-Myosin A.*† (30041)

GEORGE KALDOR AND SANDRA WEINBACH (Introduced by B. W. Volk)
*Department of Physical Chemistry, the Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.*

It had been reported previously that U.V. irradiation of myosin A led to the formation of dimers and that it also caused alterations in enzymatic activity of the protein(1). To obtain further information about the changes induced by U.V., irradiated protein was subjected to trypsin digestion and the products of this treatment were studied by ultracentrifugation, diffusion, electrophoresis and ATPase measurements.

*Materials and methods.* Myosin A was prepared as described by Mommaerts(2). The experimental techniques of ultracentrifugation, diffusion and electrophoresis, as well as ATPase determination have been described(1). U.V. irradiation was performed in a cold room (2-4°C) on a 3.2 mm thick (10 mg/ml) myosin A layer under constant slow mechanical stirring. Myosin A was dissolved in a solution containing 0.5 M KCl and 0.1 M tris buffer at pH 7.5. The output of the G15R8 General Electric germicidal lamp was measured by a dose-rate meter(3) and was found to be $8.54 \times 10^{14}$ quanta/cm$^2$ second. All samples were spun after irradiation in a Spinco model L ultracentrifuge at 20,000 RPM for 45 minutes. Trypsin digestion was performed at room temperature ($23 \pm 1$°C) for 60 minutes under constant mechanical stirring. The concentration of the myosin A was 7.5 mg/ml and that of trypsin, 0.02 mg/ml. The digestion was stopped by adding 0.03 mg/ml soybean trypsin inhibitor to the mixture. The solubility of the proteins at various ammonium sulphate concentrations was measured as published by Szent-Gyorgyi(4) and the amount of the 5% TCA soluble fraction as described by Mihalyi(5).

*Results.* After 60 minutes of trypsin digestion of native myosin, the well-known peaks of the heavy and light meromyosins were demonstrated in the analytical ultracentrifuge(5,6,7,8). If, however, the 60-minute irradiated-myosin A was digested by trypsin under identical conditions, a different ultracentrifugal pattern was obtained. Instead of 2 peaks, only one broad peak was observed which migrated with approximately the same velocity as did the slower-moving component (light meromysin) of the digested native myosin A. If actin was also added to the digested myosin (actin:myosin, 1:2, w/w) the faster moving peak disappeared. No change was observed, however, if actin was mixed in a similar proportion with the irradiated-digested myosin A. These experiments showed that while the heavy meromyosin fraction of myosin A retained, even after 60 minutes of trypsin digestion, its actin binding capacity(4,8), the split products of the irradiated-digested myosin have lost this property completely. The preservation of the actin

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† The following abbreviations have been used: U.V.-ultraviolet; TCA - trichloracetic acid; ATPase - adenosinetriphosphatase.

TABLE I. ATPase Activity and TCA Solubility of Myosin A After Various Treatments.

| Treatment of enzyme | ATPase activity in $\mu$M/mg × min | 5% TCA soluble fraction % of total |
|---|---|---|
| None | .72 | 0 |
| U.V. irradiation for 60′ | .61 | 0 |
| Trypsin digestion for 60′ | .41 | 16 |
| U.V. irradiation (60′) + trypsin digestion (60′) | .22 | 31 |

binding ability of myosin A after 60 minutes of U.V. irradiation was shown previously(1).

ATPase determinations have demonstrated (Table I) that while native myosin lost only 33% of its activity during the 60 minutes of trypsinization, the U.V. irradiated myosin lost 64% of its original activity after being exposed to trypsin under otherwise similar conditions.

The solubility of the digested and the irradiated-digested myosin A at neutral pH and at various ammonium sulphate concentrations (0-70% saturation) did not show any significant differences. They were similar to the results described by Szent-Gyorgyi with myosin A digested for 12 minutes(4). The amount of 5% TCA soluble protein was about twice as much for the irradiated-digested as for the native digested myosin A (Table I).

Further experiments were performed to obtain information about the average molecular weight of the split products of the irradiated-digested myosin A. The sedimentation constant and the diffusion constant were measured (Fig. 1) and molecular weight was calculated by the well-known Svedberg equation(9). The numerical values of the diffusion constant ($2.68 \times 10^{-7}$), sedimentation constant ($3.4 \times 10^{-13}$) and molecular weight (115,000) were close to those obtained by various authors for light meromyosin(4,10,11). In the electrophoresis experiment the irradiated-digested myosin showed a single peak with a velocity of migration of $3.05 \times 10^{-5}/\text{cm}^2 \times$ sec.

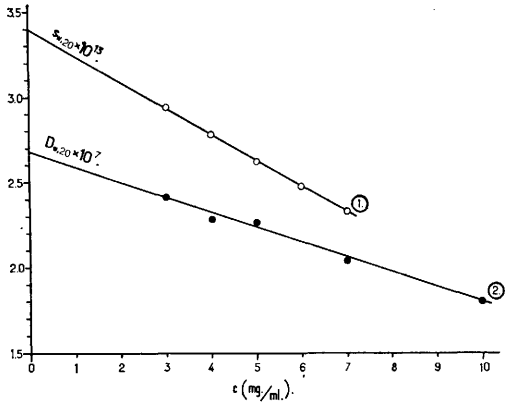


FIG. 1. Concentration dependence of sedimentation coefficient and diffusion coefficient of 60 min irradiated myosin A after 60 min of digestion. Abscissa for both curves is protein concentration in mg/ml. Ordinates for upper curve (1) is $S_{w,20} \times 10^{-13}$ and for lower curve (2) is $D_{w,20} \times 10^{-7}$.

*Discussion.* Mihalyi and Harrington(12) indicated the presence of a random chain section in the native myosin A molecule, located between the heavy and light meromyosin fractions(12). This 200-300Å long "trypsin sensitive region" was most effectively hydrolyzed by trypsin to produce the heavy and light meromyosin fragments(13). It was found previously that the 60-minute irradiated-myosin A showed an increased levorotation indicating the appearance of new random chain sections in the protein(1). The disappearance of the heavy meromyosin fraction in the ultracentrifugal pattern of the irradiated-digested myosin, as well as the loss of the actin binding capacity of this protein, suggested that U.V. irradiation induced major structural changes in the heavy meromyosin component of the myosin A molecule.

Since the ATPase activity of the irradiated-digested myosin A was also greatly reduced, one may assume that the active center of the enzyme was more exposed to hydrolysis than is the case of the native protein. It is therefore supposed that U.V. irradiation produced a second "trypsin sensitive region" on the heavy meromyosin component of the irradiated-myosin A. This, in turn, promoted the digestion of the heavy meromyosin fragment to split products of 115,000 molecular weight.

1. Kaldor, G., Gitlin, J., Westley, F., Volk, B. W., Biochemistry, 1964, v3, 1137.

2. Mommaerts, W. F. H. M., Methods Med. Res., 1958, v7, 7.

3. Jagger, J., Radiation Res., 1961, v14, 394.

4. Szent-Gyorgyi, A. G., Arch. Biochem. Biophys., 1953, v42, 305.
5. Mihalyi, E., J. Biol. Chem., 1953, v201, 197.
6. Mihalyi, E., Szent-Gyorgyi, A. G., ibid., 1953, v201, 189.
7. Gergely, J., Gouvea, M. A., Karibian, D., ibid., 1955, v212, 165.
8. Mihalyi, E., Szent-Gyorgyi, A. G., ibid., 1953, v201, 211.
9. Svedberg, T., Pedersen, K. O., The Ultracentrifuge, Oxford Univ. Press, London, 1940, p39.
10. Lowey, S., Cohen, C., J. Mol. Biol., 1962, v4, 293.
11. Young, M., Himmelfarb, S., Harrington, W. F., J. Biol. Chem., 1964, v239, 2822.
12. Mihalyi, E., Harrington, W. F., Biochim. Biophys. Acta, 1959, v36, 447.
13. Woods, E. F., Himmelfarb, S., Harrington, W. F., J. Biol. Chem., 1963, v238, 2374.

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## Plasma Lipid Variations and Fatty Acid Composition in Normal Male and Female Adult Rabbits.* (30042)

C. E. LUTTON† AND T. T. TSALTAS (Introduced by J. M. Coon)
*Department of Pathology, Jefferson Medical College, Philadelphia, Pa.*

Rabbits have been used extensively in studies on lipid metabolism and experimental atherosclerosis. Many reports in the literature present data on the plasma lipids of rabbits under a variety of experimental conditions. Few data, however, have been reported on the plasma lipid values of normal rabbits, variations in plasma lipid between male and female rabbits, or the fatty acid composition of each plasma lipid fraction(1-4). Fillios and Mann(5) reported cholesterol values to be higher in female than male rabbits, but data on other lipid fractions were not available. The data presented here deal with values of plasma lipids in normal rabbits and variations of the plasma lipids between the sexes. An analysis of the fatty acid composition of all the plasma lipid fractions in rabbits, as determined by gas liquid chromatography, is also presented.

*Experimental.* This study was performed on adult albino New Zealand rabbits weighing between 3.6 and 4.8 kg. Twenty-five male and twenty-five female rabbits were used. The animals were maintained on Purina Rabbit Chow for at least 7 days before each experiment. Food was restricted for 12 hours prior to blood-letting; water was available to the animals at all times. Blood for plasma lipid analysis was obtained from the central ear artery in a 15 ml vacutainer tube containing 0.2 ml of 5% sodium disequestrin. Blood for plasma lipid fractionation and total fatty acid analysis was obtained by exsanguinating the animals by cardiac puncture. The plasma was separated immediately from the formed elements of the blood, regardless of method of collection, by centrifugation at 4°C and 800 × *g*.

All chemicals used were of reagent grade and all solvents were redistilled prior to use. Plasma lipoprotein lipase activity (LPL) was determined by the method of Korn(6) immediately following plasma separation. Total fatty acid (TFA) analyses were carried out on chloroform:methanol (2:1) extracts of plasma as described by Albrink(7). Plasma unesterified fatty acid (UFA) concentrations were determined by the method of Dole(8). Total lipid (TL), cholesterol (C), and phospholipid (PL) concentrations were determined on aliquots obtained from Bloor's extraction of proteins and lipids precipitated by the method described by Somogyi(9). Cholesterol levels were determined by the method of Sperry and Webb(10), and phospholipid levels by the method of Stewart and Hendry (11). Total lipid concentrations were determined gravimetrically on the same extracts. Glyceride concentrations were calculated by difference from the above results.

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† Post-doctoral Fellow, U.S.P.H.S., N.I.H.